

SAN FRANCISCO **ISER**
XXI BIENNIAL MEETING
HYATT REGENCY SAN FRANCISCO AT EMBARCADERO
JULY 20–24, 2014

PROGRAM AND ABSTRACT BOOK



International Society
for Eye Research



EXPERIMENTAL EYE RESEARCH

The official journal of the International Society for Eye Research

Editor-in-Chief
Joe Hollyfield

*Cleveland Clinic Foundation,
Cleveland, OH, USA*



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for Eye Research

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WELCOME



As President, it is my great pleasure to welcome you to the XXI Biennial Meeting of the International Society for Eye Research, and to “the Golden Gate City,” San Francisco! It’s been 26 years since an ISER meeting was held in this great American city. We’re using the same conference hotel as we did back then, but many other things have changed. Our conference this year is managed by San Francisco Association Management Services, Inc. (SF AMS), which also provides our regular office support throughout the year. Special thanks go to Dr. John Penn, ISER’s Meeting Liaison, who spent countless hours helping to coordinate interactions between the SF AMS staff, Council members, and the local Program Organizers.

Key to the success of this meeting is the dedication and leadership provided by our two amazing local Program Committee Chairs—Dr. David S. Williams and Dr. Suraj Bhat, both of whom are professors at the Jules Stein Eye Institute, David Geffen School of Medicine, UCLA, Los Angeles, CA, USA. In turn, they helped to coordinate and oversee the efforts of the Section and Session Chairs, who recruited the invited speakers for the platform sessions and also selected additional speakers from the pool of submitted abstracts. Dr. Williams and Dr. Bhat also worked tirelessly with the SF AMS staff to coordinate the scheduling of the poster presentations and all of the meeting events. My most sincere thanks to them and to all who were integrally involved in the planning and execution of this meeting, for their dedicated service to ISER and to the international eye and vision research community. This promises to be a very informative and successful scientific meeting, thanks to their efforts.

ISER’s slogan is “making a world of difference in eye research.” Look around you. There are several hundred attendees at this meeting, from more than 25 countries around the globe – established investigators, new investigators, graduate and postgraduate students, some of whom are attending an ISER meeting for the first time. For some, it’s their first time being in the United States, too. We purposely strive to keep ISER meetings small in size; this format provides a greater opportunity for interaction among attendees, affords more time for people to get to most, if not all, of the posters and platform presentations that are of interest to them, and also allows for a greater percentage of platform presentations. It is integral to ISER’s mission to facilitate the attendance and participation of students and “young investigators” at our meetings. We accomplish this by providing travel awards to as many young investigators as we can. This year, we had over 100 travel award applicants; my special thanks to our Membership Committee members, who vetted these applications. Our young investigators represent the future of eye and vision research and the future of ISER.

I especially want to recognize the generous support of our corporate sponsors: Abbott Medical Optics and BrightFocus Foundation. Please take the time to thank their representatives personally for their support when you encounter them. Finally, I would also like to recognize the dedicated efforts of the ISER Council members, as well as our SF AMS team, for providing logistical planning, communications, and execution.

This is my final Biennial Meeting as ISER President. Elections for a new President will be held this fall, with a President-Elect being named by December. It's been a great honor and pleasure to serve the membership of ISER and to help chart the course of the Society over the past two years. My special thanks go out to the members of the ISER Executive Committee, Dr. Tailoi Chan-Ling (ISER Secretary), Dr. Christine McGahan (Treasurer), and Dr. John S. Penn (Meeting Liaison), who have provided invaluable counsel in the course of my tenure as President.

I wish you all a very enjoyable and productive meeting. Please take time to experience the many historic sites (and sights), cuisine, and culture of San Francisco. Catch up with long-time friends and colleagues, and make new connections and friendships.

Finally, I invite you to make plans to come to the ISER Biennial Meeting in 2016, which will be held in Tokyo, Japan!



Steven J. Fliesler, PhD
ISER PRESIDENT

WELCOME



On behalf of the International Society of Eye Research we welcome you to the XXI Biennial Meeting in the city of San Francisco, CA, USA. This meeting brings together leading basic and clinical researchers to share technical and conceptual advances in vision and ophthalmology. A vibrant scientific program, based on a free exchange of ideas and information, among experts and students, awaits delegates from all over the world. Oral and poster presentations cover a broad array of interdisciplinary subjects in clinical ophthalmology and basic sciences, from molecular assessment of fundamental visual processes that sustain the phenotype of vision to stem cells and gene therapy for the alleviation of various pathologies that impair vision.



We trust that your attendance at the ISER XXI Biennial Meeting will be highly stimulating and rewarding.

Suraj Bhat and David Williams
ISER 2014, PROGRAM COMMITTEE CHAIRS

HONORS

The International Society for Eye Research would like to acknowledge the scientists whose unrelenting research and devoted persistence have made this year's Biennial Meeting possible. Their hard work has enabled the advancement of our knowledge of the eye and vision, expanding the capabilities of the Society.

This year the Society honors John S. Penn, PhD, ISER Meeting Liaison, and David S. Williams, PhD, and Suraj P. Bhat, PhD, Host Country Program Chairs, for their outstanding organization of the XXI Biennial Meeting of the International Society for Eye Research, held in San Francisco, CA (USA), July 2014, for their exceptional contributions as the scientific program organizers, and for their support of the Biennial Meeting and the Society.

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Suraj P. Bhat, PhD (N. America) – Co-Chair, Local Organizing Committee

David S. Williams, PhD (N. America) – Co-Chair, Local Organizing Committee

HISTORY



International Society
for Eye Research

DECEMBER 1968

Fifteen scientists from eight countries met in Oxford, England, and formed a Committee to explore the possibility of establishing an international organization to support eye and vision research. Among the objectives were coordination of various channels of research communication; establishment of an international information center and clearinghouse for financial, organizational, and operational aspects of eye research; stimulation and promotion of international research cooperation; assistance and cooperation with local, national, and regional eye research organizations when needed. The first officers of the Committee were A. Pirie, Chairperson; E. A. Balazs, General Secretary; and H. Davson, Treasurer.

MARCH 1969

At a meeting held in New York, the International Committee for Eye Research was established with a membership of 50. The newly elected officers of this enlarged Committee were A. Pirie, Chairperson; N. Nordmann and G. Smelser, Vice-Chairpersons; E. A. Balazs, General Secretary; and H. Davson, Treasurer.

AUGUST 1972

N. Nordmann became Chairperson of the Committee.

SEPTEMBER 1972

International Committee for Eye Research met in Charleston, South Carolina, USA.

OCTOBER 1973

The Japanese Chapter of the International Committee for Eye Research was organized by T. Mizukawa, S. Mishima, and A. Nakajima.

MAY 1974

The International Society for Eye Research was incorporated in the State of Delaware (USA).

JUNE 1974

At the first International Congress of Eye Research (Capri, Italy), the International Committee for Eye Research was dissolved and its former members became the Board of Directors of the international Society. Subcommittees for drafting the Bylaws and designing rules for membership were established. Pro tem officers of the Society were elected: E. A. Balazs, President-Secretary; S. Dikstein, Vice President; W. J. Manski, Treasurer. National Secretaries were appointed in 22 countries as liaisons between the Society, scientists, and national organizers in the field of eye and vision research.

SEPTEMBER 1976

At the second International Congress of Eye Research (Jerusalem, Israel) the Bylaws of the International Society were discussed and finalized.

1978

The Bylaws were adopted at the meeting of the Board of Directors and new officers were elected. The Society was opened for membership applications. An annual fee for membership was collected for the first time in 1980. **New officers:** E. A. Balazs, President; J. Zadunaisky, Secretary; and K. Eakins, Treasurer.

1984

Experimental Eye Research adopted at the Congress in Alicante, Spain, as the Journal of the Society. **New officers:** J. Zadunaisky, President; J. Hollyfield, Secretary; and F. Bettelheim, Treasurer.

1988

Office of the ISER Secretariat established; J. L. Denlinger, Executive Director. **New officers:** J. Hollyfield, President; C. Belmonte, Secretary; and P. O'Brien, Treasurer.

1990

New Membership categories established: Family and Student. **New officers:** C. Belmonte, President-Elect; M. LaVail, Vice President (North America); K. Masuda, Vice President (Japan/Far East).

1992

New officers: C. Paterson, Secretary; R. Anderson, Treasurer; Luc Missotten, Vice President (Europe).

1994

New officers: C. Paterson, President-Elect; H. Mishima, Vice President (Japan/Far East); A. Milam, Vice President (North America).

1996

New officers: N. Orzalesi, Secretary; M. Burns, Treasurer; J. Tiffany, Vice President (Europe); J. Blanks, Councilor (North America); M. Riley, Councilor (North America); M. Tamai, Councilor (Japan/Far East); A. Wegener, Councilor (Europe).

1997

New Treasurer: T. Freddo.

1998

New officers: P. Kaufman, President-Elect; Y. Honda, Vice President (Japan/Far East); N. Delamere, Vice President (North America); J. Forrester, Councilor (Europe); I. Gipson, Councilor (North America).

2000

New officers: A. Bron, Secretary; A. Alm, Vice President (Europe); P. Cammarata, Councilor (North America); S. Kinoshita, Councilor (Japan/Far East).

2002

New officers: R. Anderson, President-Elect; I. Gipson, Vice President (North America); G. Duncan, Councilor (Europe); M. R. Hernandez, Councilor (North America); M. C. McGahan, Councilor (North America); M. Tamai, Vice President (Japan/Far East).

2004

New officers: N. Osborne, Vice President (Europe); J. Penn, Secretary; K. Loeffler, Councilor (Europe); S. Fliesler, Councilor (North America); N. Yoshimura, Councilor (Japan/Far East).

2006

New officers: T. Freddo, President-Elect; D. Dartt, Vice President (North America); J. McAvoy, Vice President (Pacific Rim); E. Tamm, Councilor (Europe); J. Blanks, Councilor (North America); O. Candia, Councilor (North America).

2008

New officers: M. C. McGahan, Secretary; S. Fliesler, Treasurer; O. Strauss, Vice President (Europe); N. Osborne, Councilor (Europe); T. Iwata, Councilor (Pacific Rim); J. Penn, Councilor (North America).

2010

New officers: S. Fliesler, President-Elect; A. Taylor, Vice President (North America); P. Donaldson, Vice President (Pacific Rim); J. Gallar, Councilor (Europe); C. Mitchell, Councilor (North America); S. Wilson, Councilor (North America); J. Penn, Meeting Liaison.

2012

New officers: E. Tamm, Vice President (Europe); Tailoi Chan-Ling, Secretary; M. C. McGahan, Treasurer; D. Hyde, Councilor, (North America); O. Strauss, Councilor (Europe).

2014

New officers: G. Luty, Vice President (North America); T. Iwata, Vice President (Asia Pacific); M. Karl, Young Investigator Representative (North America).

MEETINGS

The International Committee for Eye Research and the International Society for Eye Research have sponsored the following meetings:

1971

SYMPOSIUM ON LENS, Utrecht, The Netherlands

1972

SYMPOSIA ON LENS AND AGING AND TRANSPORT PROCESSES IN THE EYE, Charleston, South Carolina, USA

1974

FIRST INTERNATIONAL CONGRESS OF EYE RESEARCH, Capri, Italy

Organizers: M. deVincentis, G. Auricchio, M. Testa

1975

SYMPOSIUM ON THE PIGMENT EPITHELIUM (Proceedings of the National Eye Institute) Bethesda, Maryland, USA

1976

SECOND INTERNATIONAL CONGRESS OF EYE RESEARCH, Jerusalem, Israel

Organizer: S. Dikstein

1978

THIRD INTERNATIONAL CONGRESS OF EYE RESEARCH, Osaka, Japan

Organizer: T. Mizukawa

1980

FOURTH INTERNATIONAL CONGRESS OF EYE RESEARCH, New York, New York, USA

Organizer: E. Balazs

1982

FIFTH INTERNATIONAL CONGRESS OF EYE RESEARCH, Veldhoven, The Netherlands

Organizer: S. Bonting

1984

SIXTH INTERNATIONAL CONGRESS OF EYE RESEARCH, Alicante, Spain

Organizer: C. Belmonte

1986

SEVENTH INTERNATIONAL CONGRESS OF EYE RESEARCH, Nagoya, Japan

Organizer: S. Iwata

1988

EIGHTH INTERNATIONAL CONGRESS OF EYE RESEARCH, San Francisco, California, USA

Organizer: D. Maurice

1990

NINTH INTERNATIONAL CONGRESS OF EYE RESEARCH, Helsinki, Finland

Organizer: A. Palkama

1992

TENTH INTERNATIONAL CONGRESS OF EYE RESEARCH, Stresa, Italy

Organizer: A. Secchi

1994

ELEVENTH INTERNATIONAL CONGRESS OF EYE RESEARCH, New Delhi, India

Organizer: P. K. Khosla

1996

TWELFTH INTERNATIONAL CONGRESS OF EYE RESEARCH, Yokohama, Japan

Organizer: K. Masuda

1998

THIRTEENTH INTERNATIONAL CONGRESS OF EYE RESEARCH, Paris, France

Organizer: Y. Pouliquen

2000

FOURTEENTH INTERNATIONAL CONGRESS OF EYE RESEARCH, Santa Fe, New Mexico, USA

Organizers: N. Delamere, M. Riley

2002

FIFTEENTH INTERNATIONAL CONGRESS OF EYE RESEARCH, Geneva, Switzerland

Organizer: S. Merin

2004

SIXTEENTH INTERNATIONAL CONGRESS OF EYE RESEARCH, Sydney, Australia

Organizer: J. McAvoy

2006

SEVENTEENTH INTERNATIONAL CONGRESS OF EYE RESEARCH, Buenos Aires, Argentina

Organizer: O. Candia

2008

EIGHTEENTH INTERNATIONAL CONGRESS OF EYE RESEARCH, Beijing, China

Organizers: X. Li, J. Zhao, J. Ge, M. Lou

2010

NINETEENTH BIENNIAL MEETING OF THE INTERNATIONAL SOCIETY FOR EYE RESEARCH, Montreal, Canada

Organizers: J. Penn, M. Steinbach

2012

TWENTIETH BIENNIAL MEETING OF THE INTERNATIONAL SOCIETY FOR EYE RESEARCH, Berlin, Germany

Organizers: O. Strauss, E. Tamm

2013

SYMPOSIUM ON MOLECULAR MECHANISMS IN GLAUCOMA, Sarasota, Florida, USA

Organizer: E. Tamm

2014

TWENTY-FIRST BIENNIAL MEETING OF THE INTERNATIONAL SOCIETY FOR EYE RESEARCH, San Francisco, California, USA

Organizers: S. Bhat, D. Williams

ISER MEMBERSHIP INFORMATION

OFFICIAL JOURNAL

Experimental Eye Research (EER), published by Elsevier Ltd. (3251 Riverport Lane, St. Louis, Missouri 63043), is the official journal of the Society. A reduced subscription rate for the journal in print or electronic format is an optional membership benefit for all members. Additionally, members receive free color in the print version of *Experimental Eye Research* and free online color reproduction for all submissions, with no page charges.

In May 2014 the Society had over 750 paid members from approximately 30 countries. Membership application forms are available at the ISER exhibit. The Bylaws of the Society (Article II, Section 1) stipulate the following requirements for six membership categories:

Full Members shall be investigators who are actively engaged in eye or vision research or other fields related to eye or visual system tissues and are 7 years or more past their terminal degree.

Family Members shall be a Full Member and his/her spouse. Both Family Members will be considered "Full Members" and shall be investigators who are actively engaged in eye or vision research or other fields related to eye or visual system tissues.

Young Investigators shall be predoctoral or postdoctoral (PhD/MD/OD/DVM/DO)/equivalent students, clinical residents, or clinical fellows engaged in vision/eye research for no longer than 7 years since their terminal degree.

Sustaining Members shall be persons, organizations, societies, corporations, or agencies whose intention is to support or promote research in the broad field of eye or vision research.

Emeritus Members shall be Full Members who have 10 years' cumulative ISER membership, who have reached the age of 65, whose academic appointment is no more than 50%, and who have requested a change to Emeritus Membership in writing.

Honorary Members shall be persons who have made exceptional scientific contributions to eye or vision research or other fields related to eye or visual system tissues.

CONTACT INFORMATION

All requests for information about membership should be directed to

ISER Secretariat

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USA

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Fax: +1-415-561-8531

Email: mail@iser.org

Website: <http://www.iser.org>

Requests for information about any other matters related to the affairs of the Society should be directed to

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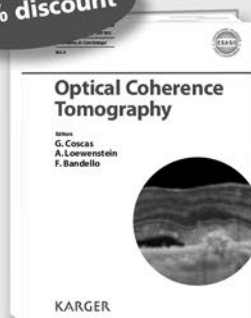
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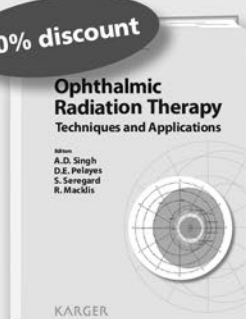
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AWARD LECTURES

THE ENDRE A. BALAZS PRIZE

This prize was made possible in part by a grant from Abbott Medical Optics

The Council of ISER awards an International Prize to honor a distinguished scientist whose outstanding contributions provide significant progress in the field of experimental eye research. This prize was named the Endre A. Balazs Prize to honor Endre A. Balazs for his distinguished work in eye research and his contributions to the organization of the International Society for Eye Research. The Endre A. Balazs Prize is awarded at every Biennial Meeting of the Society for Eye Research.

The 2014 recipient of this Prize is Patricia D'Amore. Patricia holds a PhD in Biology from Boston University, completed a postdoctoral fellowship in Biological Chemistry and Ophthalmology at Johns Hopkins Medical School, and received her MBA from Northeastern University. She is the Charles L. Schepens Professor of Ophthalmology, Professor of Pathology, Co-director of the AMD Center of Excellence, and Vice Chair of Basic Research at Harvard Medical School. She is also Director of Research, Senior Scientist, the Ankeny Scholar of Molecular Biology at the Schepens Eye Research Institute, and a Research Associate in Surgery at Boston Children's Hospital.

Dr. D'Amore has been a member of the Biological and Biomedical Sciences Program at Harvard Medical School since 1993 and has served as a Lecturer at the Marine Biology Laboratory in Woods Hole since 1994. She is a Member of The Academy at Harvard Medical School and has been recognized for her commitment to mentoring with the Everett Mendelsohn Excellence in Mentoring Award from Harvard University, the A. Clifford Barger Excellence in Mentoring Award from Harvard Medical School, and American Medical Association Women Physicians Sector Mentorship Award. She is also the recipient of the Cogan Award, and the Rous-Whipple Award and has delivered a number of named, national, and international lectures.

Dr. D'Amore's research focuses on understanding the regulation of the development and stabilization of the microvasculature. She is also investigating the pathogenesis of AMD, with a focus on inflammation. She has published 142 peer-reviewed papers in journals such as *Nature Medicine*, the *Journal of Clinical Investigation*, and *Development*, and she is the author of 66 reviews and 4 book chapters.



PREVIOUS RECIPIENTS

Hans Bloemendal (1984)
James Rae (1986)
Laszlo Bito (1988)
Anders Bill (1990)
Jose A. Zadunaisky (1992)
Joe G. Hollyfield (1994)
Elke Lütjen-Drecoll (1996)
Carlos Belmonte (1998)
Nicolas G. Bazan (2000)
Thomas Mittag (2002)
Neville Osborne (2004)
King-Wai Yau (2006)
Ilene K. Gipson (2008)
Joseph C. Besharse (2010)
Gerard A. Luttý (2012)



PREVIOUS RECIPIENTS

Johan Stjernschantz (2002)

Elke Lütjen-Drecoll (2004)

Paul Kaufman (2006)

Anthony P. Adamis (2008)

Martin B. Wax (2010)

Peter F. Kador (2012)

THE ERNST H. BÁRÁNY PRIZE

The Council of ISER awards an International Prize in honor of Professor Ernst H. Bárány, for his distinguished work in the field of ocular pharmacology. This award is presented to a distinguished scientist who has made outstanding contributions in research that increases our understanding of ocular pharmacology directly related to or applicable to glaucoma, diabetic retinopathy, macular degeneration, or related retinal diseases.

The 2014 recipient of this Prize is Thomas Yorio, Professor of Pharmacology and Neuroscience and a member of the North Texas Eye Research Institute at the University of North Texas Health Science Center. He was Founding Dean of the Graduate School of Biomedical Sciences and currently is Provost and Executive Vice President for Academic Affairs. He currently serves as Associate Editor for *IOVS* and *Journal of Ocular Pharmacology & Therapeutics*. Dr. Yorio is actively funded by the National Institutes of Health (NIH) and Department of Defense for his work in glaucoma pharmacology and currently has grants from NEI, NHLBI, NIGMS, and DoD. He has published more than 135 peer-reviewed research articles, numerous reviews, and book chapters and is Co-editor of a textbook. Dr. Yorio is an ocular pharmacologist, with an interest in glaucoma. His research over the past 3 decades has focused in the areas of ion transport in aqueous humor dynamics, endothelin pharmacology in glaucoma, and identifying potential targets for neuroprotection. Dr. Yorio has also been one of the leading investigators in the area of glucocorticoid pharmacology and ocular hypertension, specifically on understanding why glucocorticoids induce ocular hypertension in some individuals (particularly glaucoma patients) but not others. He determined, with collaborators, that the glucocorticoid receptor (GR) beta has a major role in dampening the ocular response to glucocorticoids and the lack of this receptor results in a glucocorticoid hyper-response and ocular hypertension. In addition, as an active member of ISER for more than 20 years, he has chaired or helped organize several symposia for the ISER Congress.

THE RETINA RESEARCH FOUNDATION'S PAUL KAYSER INTERNATIONAL AWARD IN RETINA RESEARCH

The Council of ISER accepted a proposal from the Retina Research Foundation (RRF), Houston, Texas, to present the Foundation's Paul Kayser International Award in Retina Research at ISER's biennial congresses beginning in 1986. Nominees for and recipients of the award are selected by Foundation officials interacting with a committee appointed by the ISER Council. Founded in 1969, Retina Research Foundation is a publicly supported, tax-exempt charitable organization that conducts an ongoing program of basic vision science research devoted to the retina and retinal diseases.

The Paul Kayser International Award in Retina Research was created by the Directors of Retina Research Foundation and endowed by the Trustees of The Kayser Foundation to honor and perpetuate the memory of longtime friend and dedicated benefactor of RRF Paul Kayser. Through this award both organizations are demonstrating the conviction they shared with Mr. Kayser that blindness caused by retinal disease is a global concern and must be addressed accordingly. It is thus the purpose of this award to foster greater awareness of the need for intensive study of the retina, its role in the visual process, and the retinal diseases that threaten and/or destroy eyesight by recognizing outstanding achievement and sustaining meritorious scientific investigations worldwide.

The 2014 recipient of this award is Robert E. Marc, a Distinguished Professor of Ophthalmology who holds the Calvin and JeNeal Hatch Presidential Chair in Ophthalmology at the University of Utah. He obtained a BSc with Honors at the University of Texas/El Paso (1971) under Jerry Hunter and a PhD in Neuroscience at the University of Texas/Houston (1975) under Harry Sperling, where they produced the first complete color maps of retinal cone arrays. Postdoctoral work with William Stell at UCLA launched a career-long interest in tracing neural pathways with molecular markers and electron microscopy, providing the first frameworks for neurochemically defined feedback systems in the retina. He joined the University of Texas/Houston as an assistant professor (1978) and became the Robert Greer Professor of Biomedical Sciences in 1986. At the University of Texas/Houston he explored retinal mapping via small molecule visualization and computational classification. In 1993 he joined the Moran Eye Center at the University of Utah. Work at Utah led to the development of computational molecular phenotyping and new probes of neuronal excitation. Using these techniques, he and Bryan Jones provided comprehensive evidence for remodeling of the neural retina in retinal degenerations. Recently, fusing computational molecular phenotyping and electron microscopy allowed Dr. Marc, Jones, Dr. J. R. Anderson, and Dr. J. S. Lauritzen to build the first 2 nm-resolution retinal connectome. His research has been funded by the NIH since 1978. In 1996, he and Ann Torrence founded Signature Immunologics, Inc. His daughter Monica Marc and grandson R. Anthony Marc live in Texas.



PREVIOUS RECIPIENTS

Shom S. Bhattacharya and
Alan F. Wright (1986)
Dennis Baylor (1988)
Berndt Ehinger and
Neville Osborne (1990)
Alan M. Laties (1992)
Alan C. Bird (1994)
Akimichi Kaneko (1996)
Anita E. Hendrickson (1998)
Debora B. Farber (2000)
Dennis M. Dacey (2002)
The research consortium
composed of Gregory Ackland,
Gustavo Aguirre,
Jean Bennett,
William Hauswirth,
Samuel Jacobson,
Albert Maguire (2004)
Dean Bok (2006)
John E. Dowling (2008)
Frank S. Werblin (2010)
Robert E. Anderson (2012)



THE LUDWIG VON SALLMANN PRIZE

Ludwig von Sallmann was a distinguished international ophthalmologist and ophthalmic investigator who served on the staffs of Vienna, Peking, and Columbia Universities and the Ophthalmology Branch of the former National Institute of Neurological Diseases and Blindness at the National Institutes of Health. His wife, Henrietta von Sallmann, established a trust fund to award, in his memory, a cash prize every two years to an individual who has distinguished himself or herself by making a significant contribution to vision research and ophthalmology.

The 2014 recipient of this Prize is Christine A. Curcio, PhD, FARVO, who has focused 30 years of research on retinal aging and age-related macular degeneration (AMD), primarily through multi-disciplinary studies of human donor eyes. Trained in neuroanatomy and morphometry, her early work on human photoreceptor and ganglion cell topography (with K.R. Sloan) are now among the most widely cited references in the vision literature and have been used to validate clinical instruments, including adaptive optics scanning laser ophthalmoscopy and rapid dark adaptometry, an early detection tool for AMD. Her contributions to AMD pathobiology include documenting that rods die before cones in aging and AMD (1990, 1993, 1996; with C. Owsley and G.R. Jackson); discovering, characterizing, and contextualizing a large age-related accumulation of lipoprotein particles of intra-ocular origin in human Bruch's membrane that constitutes the largest volumetric pathway in drusen (with M. Johnson, N. Dashti, B.H. Chung, G. Malek, C. Guidry; 1998-2011); formulating a comprehensive theory of AMD lesion pathogenesis highlighting lipoproteins (with M. Johnson, 2009-2012); and characterizing AMD-specific lesions, including basal linear deposits (1999-present) and sub-retinal drusenoid deposits (with R. Spaide, Y. Zhang, 2009-present). She recently completed NEI-funded work focused on the histological basis (cell, tissue, and subcellular) of optical coherence tomography and fundus autofluorescence, two widely used diagnostic tools for outer retinal disease. This research exploited a repository of human donor eyes accessioned over 15 years from the Alabama Eye Bank to create Project MACULA, a web-based resource for AMD pathology (<http://projectmacula.cis.uab.edu/>). Current and future research will focus on the subcellular and molecular basis of these imaging modalities plus hyperspectral autofluorescence (with R.T. Smith, T. Ach, and Z. Ablonczy, 2012-present).

PREVIOUS RECIPIENTS

Tsuneo Tomita (1984)
Gerald Westheimer (1986)
Daniel Albert (1988)
Richard F. Brubaker (1990)
John E. Dowling (1992)
Sohan Singh Hayreh (1994)
David M. Maurice (1996)
Denis A. Baylor (1998)
Helga E. Kolb (2000)
Steven K. Fisher (2002)
Jonathan Stone (2004)
Eliot Berson (2006)
Samuel Miao-Sin Wu (2008)
Robert S. Molday (2010)
Eberhardt Zrenner (2012)

TRAVEL FELLOWSHIPS

As part of its commitment to ensuring that young investigators from around the world have the opportunity to participate in its meetings, ISER underwrites a Young Investigator Travel Fellowship Program. Based upon established criteria, the ISER Travel Fellowship Committee carefully reviewed and selected 33 travel fellowship awardees from among the many deserving applicants. ISER thanks the members of the Committee for their dedicated service and congratulates all those who have received travel awards to the XXI ISER Biennial Meeting in San Francisco.

RECIPIENTS

Syed Abbas, USA	Vanessa Martino, Canada
Pachiappan Arjunan, USA	Balaraj Menon, USA
Ana Bastos-Carvalho, USA	Kacie Meyer, USA
Vicki Chrysostomou, Australia	Philip Mzyk, USA
Onkar Dhande, USA	Geraint Parfitt, USA
Rebecca Donegan, USA	Susan Shen, USA
Johanne Dubail, USA	Travis Smith, USA
Derrick Feenstra, USA	Denise Stephens, USA
Giulio Ferrari, Italy	Michael Stuck, USA
Yazad Irani, Australia	Ivan Tochitsky, USA
Mei Jiang, USA	Alex Vielma, Chile
Manuela Lahne, USA	Jennifer Williams, UK
Jacey Ma, USA	Cristina Zibetti, USA

BETTELHEIM TRAVEL FELLOWSHIP

The National Foundation for Eye Research, which established the Bettelheim Travel Fellowship, provided funds to enable young cataract researchers to participate in the XXI ISER Biennial Meeting.

RECIPIENTS

Dylan Audette, USA	Alpana Dave, Australia
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BRIGHTFOCUS FOUNDATION TRAVEL FELLOWSHIPS

The BrightFocus Foundation provided funds to enable young glaucoma and age-related macular degeneration researchers to participate in the XXI ISER Biennial Meeting.

RECIPIENTS

Barbara Maria Braunger, Germany	Sara Savage, USA
Paul Kay, United Kingdom	
Tiago Ferreira, Germany	
Daniel Ryskamp, USA	



SAN FRANCISCO **ISER**
XXI BIENNIAL MEETING
HYATT REGENCY SAN FRANCISCO AT EMBARCADERO
JULY 20–24, 2014

General Information

GENERAL INFORMATION



International Society
for Eye Research

**MARK YOUR
CALENDAR!
JUNE 6-9, 2015**



Please save the date for the 6th
WORLD GLAUCOMA CONGRESS
JUNE 6-9 2015
HONG KONG

THE GLOBAL GLAUCOMA NETWORK
WWW.WORLDGLAUCOMA.ORG

GENERAL INFORMATION

OFFICIAL LANGUAGE

The official language of the XXI Iser Biennial Meeting is English.

REGISTRATION DESK

The Registration Desk will be open during the following hours:

Sunday, July 20	16:00 – 19:00
Monday, July 21	06:30 – 18:30
Tuesday, July 22	07:30 – 18:30
Wednesday, July 23	07:30 – 12:30
Thursday, July 24	07:30 – 18:30

NAME BADGES

All participants and exhibitors are kindly requested to wear their name badges throughout the meeting in order to be admitted to the lecture halls and other scheduled activities. If you have misplaced your badge please request a replacement at the Registration Desk.

ISER BUSINESS MEETING

The Iser Business Meeting will be held Tuesday, July 22 from 11:30 – 12:00 in the Grand Ballroom following the Plenary Lecture. All Iser members are invited and encouraged to attend.

INTERNET CAFÉ

Computer stations offering participants free internet access will be located in the foyer near Registration. The stations will be available during registration hours.

EXHIBITION HOURS

The exhibition will be open as follows:

Monday, July 21	09:30 – 16:30
Tuesday, July 22	09:30 – 16:30
Wednesday, July 23	09:30 – 10:30
Thursday, July 24	09:30 – 16:30

INFORMATION BOARD

Please regularly check the information board located in the registration area for messages and important notices.

CONTINUING MEDICAL EDUCATION (CME) AND CERTIFICATE OF ATTENDANCE

CME credits are not available for the XXI Iser Biennial Meeting. Certificates of attendance will be issued to verify attendance upon request. Certificates of attendance will be available at the Registration Desk. Please ensure that you have your name badge with you.

LIABILITY AND INSURANCE

San Francisco Association Management Services (Meeting Secretariat) and the International Society for Eye Research cannot accept liability for personal accidents or loss of or damage to private property of participants and accompanying persons. Participants are advised to purchase their own personal travel and health insurance for their trip.

SAFETY AND SECURITY

Please do not leave bags or suitcases unattended at any time, whether inside or outside the session halls.

ISER MEETING SECRETARIAT

San Francisco Association
 Management Services
 655 Beach Street
 San Francisco, CA 94109
 USA

Tel: +1-415-447-0240

Fax: +1-415-561-8531

Website: www.sf-ams.com

GALA INFORMATION

San Franciscans love and take great pride in our breathtaking scenery; fresh, creative cuisine; and our baseball team. Join us at AT&T Park for a fun-filled evening of all that is quintessentially and uniquely San Francisco! Mingle in the Club Level lounge as you gaze over the field where the 2010 and 2012 World Series champions play, then get an insider's perspective as we take you on an exclusive, behind-the-scenes tour of the ballpark. Your star treatment continues when we sit down to an elegant dinner highlighting the season's bounty of the Bay Area. Afterwards, catch up with old friends and make new ones as you enjoy the musical entertainment inside – or step onto the moonlit terrace outside for beautiful, sweeping views of the Bay Bridge, San Francisco skyline and waterfront.

Inquire about availability at the Registration Desk.



EXHIBITORS

SAN FRANCISCO
ISER
XXI BIENNIAL MEETING
JULY 20–24, 2014



Association for Research in Vision and
Ophthalmology (ARVO)



Biosearch Technologies, Inc.

Center for Advanced Retinal and Therapeutic
Ophthalmics, University of Pennsylvania



Ocuscience



Powered Research, LLC

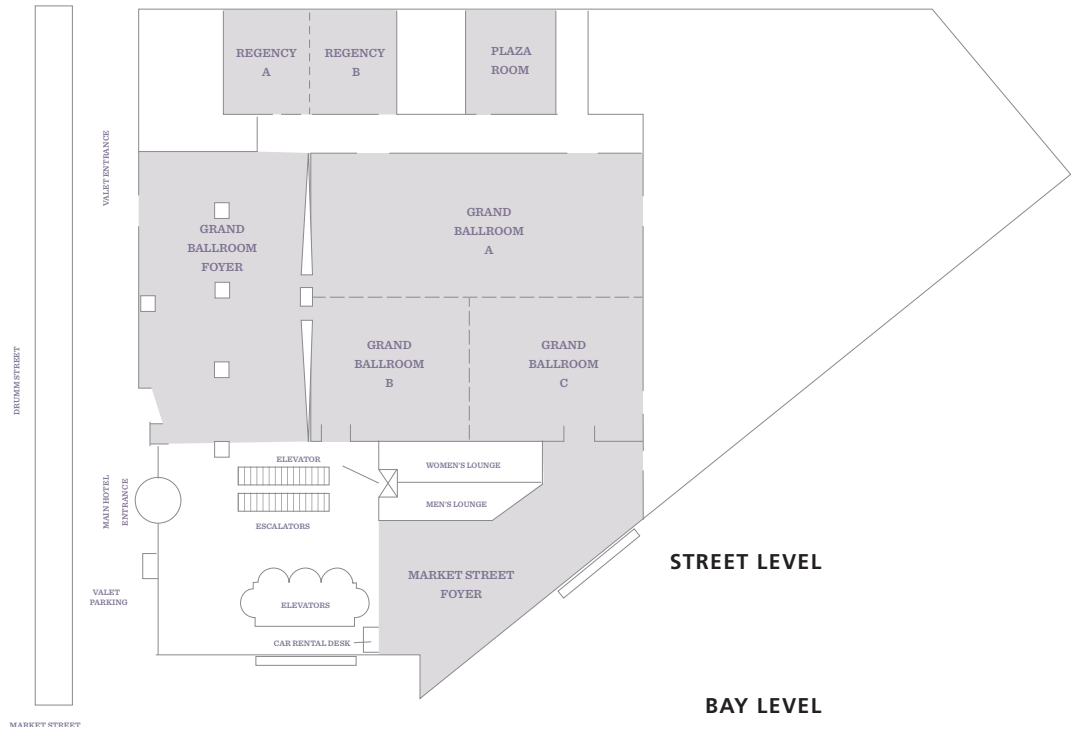


Wolters Kluwer

HOTEL MAP

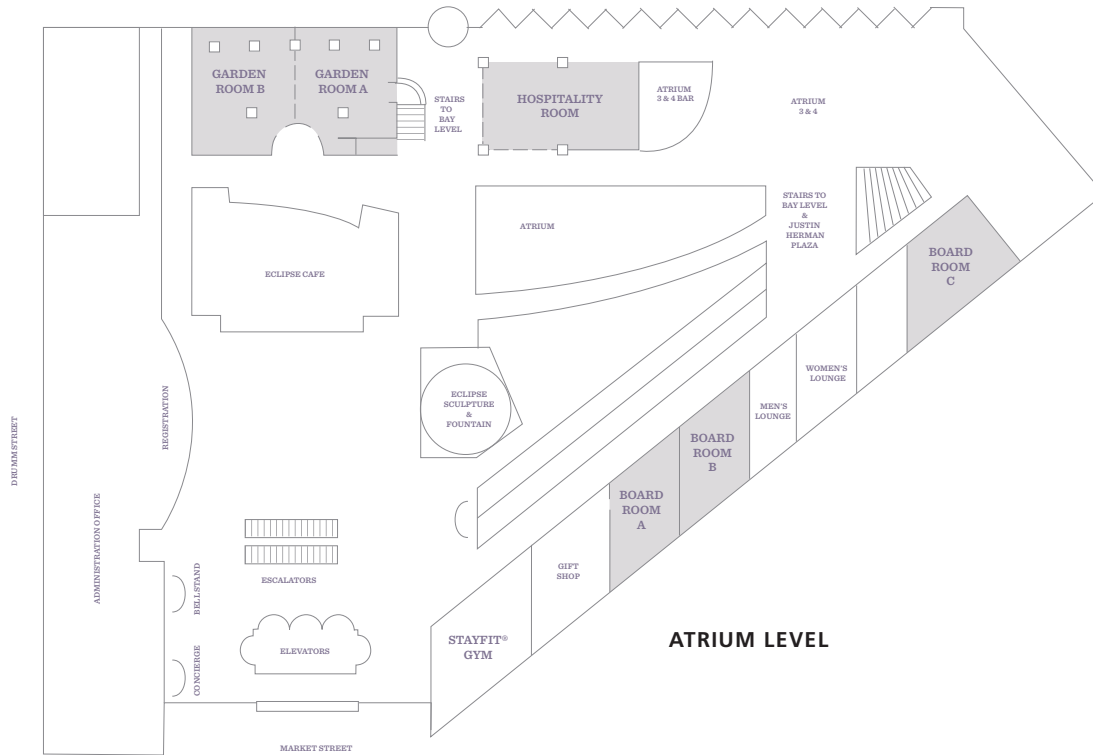
HYATT REGENCY SAN FRANCISCO (AT EMBARCADERO)

5 Embarcadero Center,
San Francisco, CA 94111
USA
Tel: +1-415-788-1234
Fax: +1-415-398-2567



HOTEL MAP

SAN FRANCISCO
ISER
XXI BIENNIAL MEETING
JULY 20-24, 2014



ATRIUM LEVEL

PACIFIC CONCOURSE LEVEL



SAN FRANCISCO **ISER**
XXI BIENNIAL MEETING
HYATT REGENCY SAN FRANCISCO AT EMBARCADERO
JULY 20–24, 2014

Schedule at a Glance

SCHEDULE AT A GLANCE



International Society
for Eye Research

SCHEDULE AT A GLANCE

SUNDAY, 20 JULY

16:00	REGISTRATION OPENS – GRAND BALLROOM FOYER
19:00	WELCOME RECEPTION – ATRIUM, HYATT REGENCY

MONDAY, 21 JULY

	CORNEA AND OCULAR SURFACE (CO)	GLAUCOMA (GL)	OCULAR IMMUNOLOGY (IM)	LENS (LE)
6:30	REGISTRATION OPENS – GRAND BALLROOM FOYER			
8:00 – 10:00	CO01 The Role of Mucins in Ocular Surface Barrier Function SEACLIFF D	GL01 Trabecular Meshwork Pathogenesis GRAND BALLROOM	IM01 Autoimmunity and Immune Privilege GOLDEN GATE	LE01 Crystallin Interactions in Health and Disease SEACLIFF A
10:00 – 10:30	COFFEE BREAK • POSTER VIEWING (ANTERIOR SEGMENT) • EXHIBITION – PACIFIC CONCOURSE			
10:30 – 12:00	OPENING CEREMONY & THE ENDRE A. BALAZS PRIZE PLENARY LECTURE – GRAND BALLROOM			
12:00 – 13:00	LUNCH BREAK • POSTER VIEWING (ANTERIOR SEGMENT) • EXHIBITION – PACIFIC CONCOURSE			
13:00 – 15:00	CO02 Immune Regulation of Cornea and Anterior Segment SEACLIFF D	GL02 Glaucomatous Optic Neuropathy GRAND BALLROOM		LE02 Can Crystallins Be Used as Therapeutic Agents? SEACLIFF A
15:00 – 16:30	COFFEE BREAK • POSTER SESSION 1 (ANTERIOR SEGMENT) • EXHIBITION – PACIFIC CONCOURSE			
16:30 – 18:30	CO03 Corneal Stem Cells and Niches SEACLIFF D	GL03 Molecular Mechanisms in Glaucomatous Neurodegeneration and Regeneration GRAND BALLROOM	IM02 Inflammatory Mediators in Age-Related Macular Degeneration GOLDEN GATE	LE03 Oxidative Stress and the Aging Lens SEACLIFF A

OCULAR IMAGING (OI)	PHYSIOLOGY AND PHARMACOLOGY (PH)	RETINAL CELL BIOLOGY (RC)	RETINAL NEURO-SCIENCE AND DEVELOPMENT (RN)	RPE BIOLOGY AND PATHOLOGY (RP)
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REGISTRATION OPENS – GRAND BALLROOM FOYER

OI01 Image-Based Outcome Measures for Treatment of Retinal Disease SEACLIFF C	PH01 Vertebrate and Invertebrate Visual Systems: Lessons from Evolution MARINA	RC01 Role of Retinal Clocks in Eye Disorders BAYVIEW B	RN01 Gene Regulation and Protein Modulation in Retinal Development SEACLIFF B	RP01 RPE Replacement and Tissue Engineering BAYVIEW A
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COFFEE BREAK • POSTER VIEWING (ANTERIOR SEGMENT) • EXHIBITION – PACIFIC CONCOURSE

OPENING CEREMONY & THE ENDRE A. BALAZS PRIZE PLENARY LECTURE – GRAND BALLROOM

LUNCH BREAK • POSTER VIEWING (ANTERIOR SEGMENT) • EXHIBITION – PACIFIC CONCOURSE

		RC02 Retinal TRP Channels and Visual Function BAYVIEW B	RN02 Development of Retinal Circuitry and Synapses SEACLIFF B	RP02 Phagosomes, Endosomes and Lipofuscin in the RPE SEACLIFF C
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COFFEE BREAK • POSTER SESSION 1 (ANTERIOR SEGMENT) • EXHIBITION

OI02 Innovations in Imaging Animal Models of Disease MARINA	PH02 Retinoids and the Vitamin A Cycle BAYVIEW A	RC03 Retinal Calcium-Binding Proteins: Structure, Function, and Role in Diseases BAYVIEW B	RN03 Mechanisms of Neuroprotection SEACLIFF B	RP03 Very Long Chain Fatty Acids in Ocular Health and Disease SEACLIFF C
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SCHEDULE AT A GLANCE

TUESDAY, 22 JULY

	CORNEA AND OCULAR SURFACE (CO)	GLAUCOMA (GL)	OCULAR IMMUNOLOGY (IM)	LENS (LE)
7:30	REGISTRATION OPENS – GRAND BALLROOM FOYER			
8:00 – 10:00	CO04 Corneal Stromal and Endothelial Biology SEACLIFF D	GL04 Glaucoma Genetics GRAND BALLROOM	IM03 New Insights into the Immunology of Ocular Tumors GOLDEN GATE	LE04 Communica- tion in the Eye: Channels and Transporters SEACLIFF A
10:00 – 10:30	COFFEE BREAK • POSTER VIEWING (POSTERIOR SEGMENT) • EXHIBITION – PACIFIC CONCOURSE			
10:30 – 11:45	THE ERNST H. BÁRÁNY PRIZE PLENARY LECTURE – GRAND BALLROOM			
11:45 – 13:00	BUSINESS MEETING • LUNCH BREAK • POSTER VIEWING (POSTERIOR SEGMENT) • EXHIBITION – PACIFIC CONCOURSE			
13:00 – 15:00	CO05 Corneal Development, Differentiation, and Genetics SEACLIFF D	GL05 Animal Models of Glaucoma GRAND BALLROOM		LE05 Cataract Genetics and Genomics SEACLIFF A
15:00 – 16:30	COFFEE BREAK • POSTER SESSION 2 (POSTERIOR SEGMENT) • EXHIBITION – PACIFIC CONCOURSE			
16:30 – 18:30	CO06 Corneal Innervation: Lesson from the Basic Science SEACLIFF D	GL06 Biomechanics in Glaucoma: Trabecular Meshwork and Optic Nerve Head GRAND BALLROOM		LE06 Death and Differentiation: Novel Pathways in the Lens SEACLIFF A
19:30	GALA BANQUET			

OCULAR IMAGING (OI)	PHYSIOLOGY AND PHARMACOLOGY (PH)	RETINAL CELL BIOLOGY (RC)	RETINAL NEURO- SCIENCE AND DEVELOPMENT (RN)	RPE BIOLOGY AND PATHOLOGY (RP)
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REGISTRATION OPENS – GRAND BALLROOM FOYER

OI03 Imaging Struc- ture and Func- tion in Glaucoma SEACLIFF C	PH03 Photoreceptor Physiology and Adaptation MARINA	RC04 Retinal Angiogenesis BAYVIEW B	RN04 Glial Cells in Retina Regeneration and Repair SEACLIFF B	RP04 RPE and the Complement System BAYVIEW A
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COFFEE BREAK • POSTER VIEWING (POSTERIOR SEGMENT) • EXHIBITION – PACIFIC CONCOURSE

THE ERNST H. BÁRÁNY PRIZE PLENARY LECTURE – GRAND BALLROOM

BUSINESS MEETING • LUNCH BREAK • POSTER VIEWING (POSTERIOR SEGMENT) • EXHIBITION – PACIFIC CONCOURSE

		RC05 Photoreceptor Cell Biology I BAYVIEW A	RN05 RGC Develop- ment, Survival, and Axonal Guidance SEACLIFF B	RP05 Choroid in Health and AMD SEACLIFF C
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COFFEE BREAK • POSTER SESSION 2 (POSTERIOR SEGMENT) • EXHIBITION – PACIFIC CONCOURSE

OI04 New Approaches to Imaging Retinal and Choroidal Vasculature SEACLIFF B	PH04 Defects in Ion Channel Function Causing Disease MARINA	RC06 Role of Mononuclear Phagocytes in Age-Related Macular Degeneration BAYVIEW B	RN06 Road to Cure I: Stem Cell and ES/iPs Cell Therapy BAYVIEW A	RP06 Signaling Pathways and Organelle Dynamics SEACLIFF C
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GALA BANQUET

SCHEDULE AT A GLANCE

WEDNESDAY, 23 JULY

	CORNEA AND OCULAR SURFACE (CO)	GLAUCOMA (GL)	OCULAR IMMUNOLOGY (IM)	LENS (LE)
7:30	REGISTRATION OPENS – GRAND BALLROOM FOYER			
8:00 – 10:00	CO07 Corneal Angiogenesis from Translational Aspects SEACLIFF D	GL07 Blood Flow in Glaucoma GRAND BALLROOM	IM04 Ocular Infection and Host Defense GOLDEN GATE	LE07 Lens Development SEACLIFF A
10:00 – 10:30	COFFEE BREAK • POSTER VIEWING (ANTERIOR / POSTERIOR SEGMENT) • EXHIBITION – PACIFIC CONCOURSE			
10:30 – 11:45	THE RETINA RESEARCH FOUNDATION'S PAUL KAYSER INTERNATIONAL AWARD IN RETINA RESEARCH PLENARY LECTURE – GRAND BALLROOM			
FREE TIME	EXPLORE THE CITY			

OCULAR IMAGING (OI)	PHYSIOLOGY AND PHARMACOLOGY (PH)	RETINAL CELL BIOLOGY (RC)	RETINAL NEURO- SCIENCE AND DEVELOPMENT (RN)	JOINT SESSION (JT)
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REGISTRATION OPENS – GRAND BALLROOM FOYER

OI05 Fusion of Imaging and Bioengineering for Eye Disease MARINA	PH05 Progress in Human Gene Therapy BAYVIEW A	RC07 Unfolded Protein Response in Ox- idative Stress in Retinal Degener- ations BAYVIEW B	RN07 Cell-to-Cell Signaling and Retinal Development SEACLIFF B	JT01 Joint Session on RPE and Photoreceptor Biology SEACLIFF C
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COFFEE BREAK • POSTER VIEWING (ANTERIOR / POSTERIOR SEGMENT) • EXHIBITION – PACIFIC CONCOURSE

THE RETINA RESEARCH FOUNDATION'S PAUL KAYSER INTERNATIONAL AWARD IN
 RETINA RESEARCH PLENARY LECTURE – GRAND BALLROOM

EXPLORE THE CITY



SCHEDULE AT A GLANCE

THURSDAY, 24 JULY

	CORNEA AND OCULAR SURFACE (CO)	GLAUCOMA (GL)	OCULAR IMMUNOLOGY (IM)	LENS (LE)
7:30	REGISTRATION OPENS – GRAND BALLROOM FOYER			
8:00 – 10:00	CO08 Novel Corneal Imaging Technologies from Basic Science to Clinical Application SEACLIFF D	GL08 Aqueous Humor Dynamics GRAND BALLROOM	IM05 Pathogenic Antibodies in Ocular Disease SEACLIFF C	LE08 Signaling Pathways Determining Lens Cell Fate and Differentiation SEACLIFF A
10:00 – 10:30	COFFEE BREAK • POSTER VIEWING (ANTERIOR / POSTERIOR SEGMENT) • EXHIBITION – PACIFIC CONCOURSE			
10:30 – 11:45	THE LUDWIG VON SALLMANN PRIZE PLENARY LECTURE – GRAND BALLROOM			
11:45 – 13:00	LUNCH BREAK • POSTER VIEWING (ANTERIOR / POSTERIOR SEGMENT) • EXHIBITION – PACIFIC CONCOURSE			
13:00 – 15:00	CO09 Corneal Tissue Engineering and Gene Therapy SEACLIFF D	GL09 IOP-Lowering Agents GRAND BALLROOM		LE09 PCO: Can We Prevent Secondary Cataract? SEACLIFF A
15:00 – 16:30	COFFEE BREAK • POSTER SESSION 3 (ANTERIOR/POSTERIOR) • EXHIBITION – PACIFIC CONCOURSE			
16:30 – 18:30	CO10 Somatic and Pluripotent Stem Cell Technology: Towards the Corneal Regeneration SEACLIFF D	GL10 Hot Topics in Glaucoma GRAND BALLROOM		LE10 Molecular Basis for Lens Architecture and Functional Quality SEACLIFF A

PHYSIOLOGY AND PHARMACOLOGY (PH)	RETINAL CELL BIOLOGY (RC)	RETINAL NEURO-SCIENCE AND DEVELOPMENT (RN)	RPE BIOLOGY AND PATHOLOGY (RP)	JOINT SESSION (JT)
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REGISTRATION OPENS – GRAND BALLROOM FOYER

PH06 Novel Engineered Therapies for Glaucoma MARINA	RC08 Photoreceptor Cell Biology II BAYVIEW B	RN08 Retinal Degeneration Genetics and Mechanisms SEACLIFF B	RP07 NLRP3 Inflammasome BAYVIEW A	
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COFFEE BREAK • POSTER VIEWING (ANTERIOR / POSTERIOR SEGMENT) • EXHIBITION – PACIFIC CONCOURSE

THE LUDWIG VON SALLMANN PRIZE PLENARY LECTURE – GRAND BALLROOM

LUNCH BREAK • POSTER VIEWING (ANTERIOR / POSTERIOR SEGMENT) • EXHIBITION – PACIFIC CONCOURSE

	RC09 Cell Polarity and Signaling during Ocular Development BAYVIEW B	RN09 Epigenetics in Development and Diseases SEACLIFF B	RP08 AMD Genetics, Pathogenesis, and Stem Cells BAYVIEW A	JT02 Ion Channels in Eye Disease SEACLIFF C
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COFFEE BREAK • POSTER SESSION 3 (ANTERIOR/POSTERIOR) • EXHIBITION – PACIFIC CONCOURSE

	RC10 Signaling Sphingolipids in Ocular Diseases BAYVIEW B	RN10 Road to Cure II: Gene Replacement, Optogenetics, and Other Therapies for Retinal Diseases SEACLIFF B	RP09 Generating RPE from Stem Cells BAYVIEW A	JT03 The New Concept of the Blood-Aqueous Barrier SEACLIFF C
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SAN FRANCISCO **ISER**
XXI BIENNIAL MEETING
HYATT REGENCY SAN FRANCISCO AT EMBARCADERO
JULY 20–24, 2014



Schedule – Oral
Presentations

SCHEDULE – ORAL PRESENTATIONS



International Society
for Eye Research

Information for Scientific Sessions

The scientific program for the XXI Biennial Meeting will be composed of plenary lectures, symposia, and poster presentations. Sessions will take place in different rooms simultaneously each day. Please check the program for the room names and times of daily sessions and meeting activities.

Plenary Lectures will be presented at the following times in the Grand Ballroom:

Monday, 21 July 10:30 – 11:45

The Endre A. Balazs Prize Plenary Lecture

RECIPIENT: Patricia A. D'Amore, PhD, MBA

PRESENTER: Gerard A. Luttý, PhD

TITLE: The Role of VEGF in Vascular Growth and Stability

Tuesday, 22 July 10:30 – 11:45

The Ernst H. Bárány Prize Plenary Lecture

RECIPIENT: Thomas Yorio, PhD

PRESENTER: Ganesh Prasanna, PhD

TITLE: Glucocorticoid Receptors and Ocular Hypertension: A Question of Balance

Wednesday, 23 July 10:30 – 11:45

**The Retina Research Foundation's Paul Kayser International Award
in Retina Research Plenary Lecture**

RECIPIENT: Robert E. Marc, PhD

PRESENTER: Steven J. Fliesler, PhD

TITLE: Mapping Retinal Cells and Networks

Thursday, 24 July 10:30 – 11:45

The Ludwig von Sallmann Prize Plenary Lecture

RECIPIENT: Christine A. Curcio, PhD, FARVO

PRESENTER: James Handa, MD

TITLE: What the Photoreceptors Taught Us About Their Support System in Aging
and Age-Related Macular Degeneration

Monday, 21 July 2014

Lens

08:00 – 10:00 LE01 – Crystallin Interactions in Health and Disease

Seacliff A

Moderators: Jayanti Pande, C. Mohan Rao

- 08:00 CRYSTALLIN INTERACTIONS IN HEALTH AND DISEASE
JAYANTI PANDE
- 08:05 DYNAMIC INTERACTIONS OF α B-CRYSTALLIN
JUSTIN BENESCH
- 08:24 THE INTERACTION OF ALPHA-CRYSTALLIN WITH DISEASE-RELATED,
AGGREGATING PROTEINS
JOHN CARVER
- 08:43 FUNCTIONAL ELEMENTS IN HUMAN α B CRYSTALLIN
JOHN CLARK
- 09:02 COPPER BINDING AND CYTOPROTECTION: ALPHA-CRYSTALLIN AND OTHER SMALL HEAT SHOCK
PROTEINS
CHINTALAGIRI MOHAN RAO
- 09:21 AGE-RELATED CROSSLINKING IN HUMAN LENS PROTEINS: REVELATIONS ON MECHANISMS AND
LENS PROTEIN PACKING
KEVIN SCHEY, Zhen Wang
- 09:40 ALPHA-A CRYSTALLIN-DERIVED PEPTIDES: POTENTIAL THERAPEUTIC CHAPERONES
KRISHNA SHARMA, Murugesan Raju, Puttur Santhoshkumar

13:00 – 15:00 LE02 – Can Crystallins Be Used as Therapeutic Agents?

Seacliff A

Moderators: Mark Petrash, Kirsten Lampi

- 13:05 FORMULATION OF CRYSTALLINS AS NANOMEDICINES
ARUN UPADHYAY, Niklaus Mueller, Mark Petrash, Uday Kompella
- 13:27 THERAPEUTIC POTENTIAL OF SHSP PEPTIDES IN EYE DISEASES
RAM NAGARAJ, Sruthi Sampathkumar, Rooban Nahomi
- 13:49 MECHANISMS OF AXONAL REGENERATION IN POSTNATAL AND ADULT MONKEY RETINAL
GANGLION CELLS
SOLON THANOS
- 14:11 PEPTIDE APTAMERS: POTENTIAL TOOLS TO NEGATIVELY OR POSITIVELY MODULATE HSP27
(HSPB1) FUNCTIONS
ANDRE PATRICK ARRIGO
- 14:33 SMALL HEAT SHOCK PROTEINS ARE EFFECTIVE IN NEUROINFLAMMATORY AND ISCHEMIC
DISEASE MODELS
LAWRENCE STEINMAN

16:30 – 18:30 LE03 – Oxidative Stress and the Aging Lens

Seacliff A

Moderators: Julie Lim, Marjorie Lou

- 16:30** **BIOCHEMICAL CHANGES IN BOVINE LENSES EXPOSED TO HYPERBARIC OXYGEN: IS IT A GOOD MODEL FOR MIMICKING AGE RELATED NUCLEAR CATARACTS?**
JULIE LIM, Bo Li, Paul Donaldson
- 16:54** **IDENTIFICATION OF CRYSTALLIN DISULFIDE BONDS IN CATARACTOUS HUMAN LENSES**
LARRY DAVID, Phillip Wilmarth, Eileen Yue
- 17:18** **PARKIN-DIRECTED MITOPHAGY GOVERNS LENS EPITHELIAL CELL MITOCHONDRIAL DEGRADATION UNDER OXIDATIVE STRESS CONDITIONS**
LISA BRENNAN, Daniel Chauss, Marc Kantorow
- 17:42** **THE THIOL OXIDATION REPAIR ENZYME, GLUTAREDOXIN, CONTROLS SENILE CATARACT FORMATION**
MARJORIE LOU
- 18:06** **MULTIFUNCTIONAL ANTIOXIDANTS FOR THE TREATMENT OF AGE RELATED OXIDATIVE CHANGES IN THE LENS AND RETINA**
PETER KADOR, Hiroyoshi Kawada, James Randazzo

Glaucoma

08:00 – 10:00 GL01 – Trabecular Meshwork Pathogenesis

Grand Ballroom

Moderators: Douglas Rhee, Ernst Tamm

- 08:00** **ALTERED MECHANOBIOLOGY OF SCHLEMM'S CANAL ENDOTHELIUM IN GLAUCOMA**
DARRYL OVERBY, Enhua Zhou, Rudolf Fuchshofer, Ryan Pedrigi, Rocio Vargas-Pinto, Amir Vahabikashi, Sietse Braakman, Ritika Gupta, Kristin Perkumas, Daniel Stamer, C. Ross Ethier, Jeffrey Fredberg, Mark Johnson
- 08:24** **NOVEL CHARACTERIZATION AND LIVE IMAGING OF PROX-1 EXPRESSING SCHLEMM'S CANAL**
TAN TRUONG, Hannah Li, Young-Kwon Hong, Chen Lu
- 08:48** **RE-EXAMINING THE MONKEY OCULAR HYPERTENSION MODEL**
PAUL RUSSELL, Joshua Morgan, Vijay Raghunathan, T. Michael Nork, Brian Christian, Richard Dubielzig, Christopher Murphy
- 09:12** **DECORIN, A MODULATOR OF TGF- β 2 AND CTGF SIGNALING IN THE TRABECULAR MESHWORK**
RUDOLF FUCHSHOFER
- 09:36** **ROLE OF SPARC IN IOP REGULATION**
DOUGLAS RHEE

13:00 – 15:00 GL02 – Glaucomatous Optic Neuropathy

Grand Ballroom

Moderators: James Morgan, Nick Marsh-Armstrong

- 13:00** **RADIATION PRETREATMENT, OPTIC NERVE HEAD CELLULAR RESPONSES AND OPTIC NERVE INJURY IN AN EXPERIMENTAL RAT GLAUCOMA MODEL**
ELAINE JOHNSON, William Cepurna, Tiffany Choe, John Morrison
- 13:20** **ASTROCYTE REACTIONS IN RESPONSE TO ELEVATED INTRAOCULAR PRESSURE**
TATJANA JAKOBS

13:40	TARGETING NEUROINFLAMMATION TO COMBAT GLAUCOMA GARETH HOWELL, Ileana Soto, Richard Libby, Michael Anderson, Simon John
14:00	MITOCHONDRIAL ENERGETIC IMPAIRMENT IN GLAUCOMA IAN TROUNCE, Nicole Van Bergen, Vicki Chrysostomou, Kathryn Burdon, Shiwani Sharma, Alex Hewitt, Jaime Craig, Jonathan Crowston
14:20	THE LAMINA CRIBROSA REGION OF THE OPTIC NERVE HEAD IN A SPONTANEOUS FELINE GENETIC MODEL OF GLAUCOMA GILLIAN MCLELLAN, N. Matthew Ellinwood, Leandro Teixeira, Forest Danford, Jonathan Vande Geest
14:40	ACTIN-RICH ASTROCYTIC PROCESSES WITHIN THE OPTIC NERVE HEAD REORIENT EARLY AND SPECIFICALLY IN RESPONSE TO ELEVATED INTRAOCULAR PRESSURE SHANDIZ TEHRANI, Elaine Johnson, William Cepurna, John Morrison

16:30 – 18:30	GL03 – Molecular Mechanisms in Glaucomatous Neurodegeneration and Regeneration Moderators: Richard Libby, Dietmar Fischer	Grand Ballroom
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16:30	TRANSITIONING REGENERATIVE THERAPIES FROM THE BENCH TO THE CLINIC JEFFREY GOLDBERG
16:55	COMBINATORIAL APPROACHES FOR OPTIC NERVE REPAIR DIETMAR FISCHER
17:20	EXERCISE REVERSES FUNCTIONAL AND STRUCTURAL LOSS AFTER OPTIC NERVE INJURY: MEDIATION BY BRAIN-DERIVED NEUROTROPHIC FACTOR VICKI CHRYSOSTOMOU, Ian Trounce, Eamonn Fahy, Jonathan Crowston
17:40	STRATEGIES FOR ENHANCING NEURONAL SURVIVAL FOLLOWING OPTIC NERVE INJURY JOSEPH LEWCOCK
18:05	DISTINCT SIGNALING PATHWAYS CONTROL COMPARTMENTALIZED RETINAL GANGLION CELL DEGENERATION AFTER AXONAL INJURY RICHARD LIBBY, Kimberly Fernandes, Jeffrey Harder, Peter Shrager, Simon John

Cornea and Ocular Surface

08:00 – 10:00	C001 – The Role of Mucins in Ocular Surface Barrier Function Moderators: Ilene Gipson, Pablo Argüeso	Seacliff D
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08:00	COMPARISON OF THE TRANSMEMBRANE MUCINS MUC1 AND MUC16 IN HUMAN CORNEAL EPITHELIAL BARRIER FUNCTION ILENE GIPSON
08:20	GALECTIN-MUCIN INTERACTIONS IN OCULAR SURFACE HEALTH AND DISEASE PABLO ARGÜESO
08:40	CLUSTERIN (CLU) PREVENTS OCULAR SURFACE DAMAGE IN A MOUSE MODEL FOR HUMAN DRY EYE DISEASE SHINWU JEONG, Aditi Bauskar, M. Elizabeth Fini

09:00 **ROLE OF THE MEMBRANE-ANCHORED MUCINS MUC1 AND MUC16 IN SUPPRESSING TOLL-LIKE RECEPTOR MEDIATED INNATE IMMUNE RESPONSES AT THE OCULAR SURFACE**
BALARAJ MENON, Christina Marko, Sandra Spurr-Michaud, Ann Tisdale, Ilene Gipson

09:20 **ALTERED MUCIN GLYCOSYLATION IN THE PATHOGENESIS OF AQUEOUS-DEFICIENT DRY EYE**
DENISE STEPHENS, Trinkia Vijmasi, Nancy McNamara

13:00 – 15:00 CO02 – Immune Regulation of Cornea and Anterior Segment Seacliff D
Moderators: Reza Dana, Jerry Niederkorn

13:00 **DYSREGULATION OF TREGS IN CORNEAL INFLAMMATION**
REZA DANA, Jing Hua, Yihe Chen

13:17 **THE TWO FACES OF INTERFERON- γ IN CORNEAL TRANSPLANT SURVIVAL AND REJECTION**
JERRY NIEDERKORN, Krishen Cunnusamy

13:34 **IS CONJUNCTIVA-ASSOCIATED LYMPHOID TISSUE INVOLVED IN OCULAR SURFACE IMMUNE REGULATION?**
PHILIPP STEVEN, Sebastian Siebelmann, Sebastian Schwab, Michael Stern, Jerry Niederkorn, Uta Gehlsen

13:51 **REGULATION OF OCULAR INFLAMMATION BY MESENCHYMAL STEM CELLS**
SUNIL CHAUHAN

14:08 **OCULAR NEUROPEPTIDE REGULATION OF INNATE IMMUNITY AND ANTIGEN PRESENTATION**
ANDREW TAYLOR

14:25 **REGULATORY PMN CONTROL T CELL RESPONSE SEX- SPECIFICALLY THROUGH LIPOXIN A4-MEDIATED PROCESS**
YUAN GAO, Kyungji Min, Matthew Greenwood, Jonanthan Jong, Karsten Gronert

14:35 **HLA-A*0206 WITH TLR3 POLYMORPHISM EXERTS MORE THAN ADDITIVE EFFECTS IN COLD MEDICINE RELATED STEVENS-JOHNSON SYNDROME WITH SEVERE OCULAR COMPLICATIONS**
MAYUMI UETA, Katsushi Tokunaga, Chie Sotozono, Hiromi Sawai, Shigeru Kinoshita

14:45 **MOLECULAR MECHANISMS OF IMMUNE PRIVILEGE OF THE CORNEAL ALLOGRAFT**
MACHIKO SHIMMURA-TOMITA

16:30 – 18:30 CO03 – Corneal Stem Cells and Niches Seacliff D
Moderators: Friedrich Kruse, Mary Ann Stepp

16:30 **SOX9 AND PPAR GAMMA AS POTENTIAL REGULATORS OF CORNEAL EPITHELIAL DIFFERENTIATION**
FRIEDRICH KRUSE, Johannes Menzel-Severing, E. Meyer, U. Schlotzer-Schrehardt

16:45 **LRIG1 CONTROLS CORNEAL MAINTENANCE THROUGH THE STAT3-DEPENDENT INFLAMMATORY PATHWAY**
TAKAHIRO NAKAMURA

17:00 **INHIBITION OF TGF β SIGNAL TRANSDUCTION FACILITATES LIMBAL EXPLANT OUTGROWTH CULTURE IN DEFINED, XENO-FREE MEDIUM**
J. MARIO WOLOSIN, Aldo Zamudio, Zheg Wang

17:15 **SEEING AROUND THE CORNER AT FUTURE STRATEGIES IN CORNEAL REGENERATION**
ANDREW HOPKINSON, Matthew Branch, Laura Sidney, Samantha Wilson, Harminder Dua

17:30	ALTERATION IN LACRIMAL GLAND CELLULAR TURNOVER AND PROGENITOR CELL POPULATION IN A MOUSE MODEL OF DRY EYE DARLENE DARTT, Marie Shatos, Robin Hodges, Bruce Turpie, Sharmila Masli
17:45	CORNEAL STEM CELL DERIVED GOBLET CELLS AND WNT SIGNALING PATHWAY AHDEAH PAJOOHESH-GANJI, Sonali Pal-Ghosh, Gauri Tadvalkar, Mary Ann Stepp
18:00	INSULIN-LIKE GROWTH FACTOR-1 SUPPORTS DIFFERENTIATION AND TRANSDIFFERENTIATION OF STEM CELLS INTO CORNEAL-LIKE EPITHELIAL CELLS PETER TROSAN, Eliska Javorkova, Alena Zajicova
18:10	THE LANTS, A NEW FAMILY OF EXTRACELLULAR REGULATORS OF LIMBAL EPITHELIAL BEHAVIOUR KEVIN HAMILL, Valentina Iorio, Lee Troughton

Ocular Immunology

08:00 – 10:00 **IM01 – Autoimmunity and Immune Privilege**

Golden Gate

Moderator: Rachel Caspi

08:05	COMMENSAL MICROBIOTA AS A TRIGGER OF AUTOIMMUNE UVEITIS RACHEL CASPI, Reiko Horai, Carlos Zárate-Bladés, Jun Chen, Phyllis Silver, Patricia Dillenburg-Pilla, Yingyos Jittayasothorn, Chi-Chao Chan, Hidehiro Yamane, Kenya Honda
08:30	ANALYSIS OF RETINAL ANTIGEN-SPECIFIC TREGS AND DENDRITIC CELLS IN RETINAL T CELL RESPONSES AND EAU DALE GREGERSON, Scott McPherson, Neal Heuss, Mark Pierson
08:55	AUTOIMMUNE MECHANISMS IN AGE RELATED MACULAR DEGENERATION VICTOR PEREZ, Ali Saeed, Fernando Cruz Guilloty
09:20	CORNEAL CELL-MEDIATED IMMUNE REGULATION AND IMMUNE PRIVILEGE JUNKO HORI
09:45	RESTORING IMMUNOSUPPRESSIVE FUNCTION OF REGULATORY T CELLS REDUCES CORNEAL INFLAMMATION IN HIGH RISK CORNEAL TRANSPLANTATION MARYAM TAHVILDARI, Masahiro Omoto, Yihe Chen, Sunil Chauhan, Reza Dana

16:30 – 18:30 **IM02 – Inflammatory Mediators in Age-Related Macular Degeneration**

Golden Gate

Moderator: Sinha Debasish

16:35	MICROGLIA / MACROPHAGE BEHAVIOR DURING AGE-RELATED MACULAR DEGENERATION ANDREW DICK
16:56	THE ALTERNATIVE PATHWAY OF COMPLEMENT IN AGE-RELATED MACULAR DEGENERATION BAERBEL ROHRER
17:17	ACTIVATION OF INNATE IMMUNE SYSTEM VIA PATTERN RECOGNITION RECEPTORS IN AGE-RELATED MACULAR DEGENERATION KAI KAARNIRANTA

17:38	DYSREGULATED LYSOSOMAL-MEDIATED CLEARANCE SHIFTS PARA-INFLAMMATION TO CHRONIC INFLAMMATION IN RPE CELLS DEBASISH SINHA, Mallika Valapala, Rhonda Grebe, Imran Bhutto, Stacey Hose, Marisol Cano, Eric Wawrousek, James Handa, Gerard Luty, J. Samuel Zigler Jr.
17:59	CIGARETTE SMOKE (CS), CYTOPROTECTIVE NRF2 SIGNALING AND THE INNATE IMMUNE RESPONSE DURING AGE-RELATED MACULAR DEGENERATION (AMD) JAMES HANDA, Marisol Cano, Lei Wang, Katayoon Ebrahimi, Hong Wei

Physiology and Pharmacology

08:00 – 10:00 **PH01 – Vertebrate and Invertebrate Visual Systems: Lessons from Evolution** Marina

Moderator: Nansi Colley

08:00	THE EVOLUTIONARILY CONSERVED SNARE PROTEIN, GOS28, IN DROSOPHILA NANSI COLLEY, Erica Rosenbaum, Eva Vasiljevic, Spencer Cleland, Carlos Flores
08:30	EVOLUTION OF TRP CHANNELS: A LESSON FROM MUTATION AND CONSERVATION OF <i>DROSOPHILA</i> TRPL PORE DOMAIN BARUCH MINKE, Maximilian Peters
09:00	A JOURNEY TO THE EVOLUTIONARY ORIGINS OF ANIMAL VISION TODD OAKLEY
09:30	EVOLUTION OF VERTEBRATE PHOTOTRANSDUCTION AND THE RETINOID CYCLE: CLUES FROM AGNATHANS TREVOR LAMB, Aaron Chuah, Hardip Patel, David Hunt

16:30 – 18:30 **PH02 – Retinoids and the Vitamin A Cycle** Bayview A

Moderator: John Nickerson

16:35	IRBP AND THE VERTEBRATE RETINA JOHN NICKERSON
16:58	STRUCTURAL AND FUNCTIONAL BASIS OF RPE65 CATALYTIC MECHANISM T. MICHAEL REDMOND, Tingting Liu, Abdulkerim Eroglu, Eugenia Poliakov, Susan Gentleman
17:21	A2E AND LIPOFUSCIN ROSALIE CROUCH, Yiannis Koutalos, Kevin Schey, Zsolt Ablonczy
17:44	ATOMIC STRUCTURES OF THE RETINOID CYCLE PROCESSING ENZYMES KRZYSZTOF PALCZEWSKI
18:07	CAN RODS REGENERATE THEIR PIGMENT WITHOUT THE RPE? VLADIMIR KEFALOV

Ocular Imaging

08:00 – 10:00 **OI01 – Image-Based Outcome Measures for Treatment of Retinal Disease**

Moderator: Mark Pennesi

Seacliff C

- 08:05 **FUNDUS AUTOFLUORESCENCE IMAGING**
MICHEL MICHAELIDES
- 08:28 **OPTICAL COHERENCE TOMOGRAPHY PATTERN OF CENTRAL PHOTORECEPTOR IMPAIRMENT IN PATIENTS WITH RETINITIS PIGMENTOSA**
BARBARA DELAS, Gemma Julio, Sara Lluch, Maria Alcala, Mouafk Asaad
- 08:51 **ELLIPSOID ZONE WIDTH ANALYSIS OF OCT**
DAVID BIRCH, Donald Hood, Dianna Wheaton, Dennis Hoffman
- 09:14 **REAL-TIME MONTAGING OF ADAPTIVE OPTICS RETINAL IMAGES**
TRAVIS SMITH
- 09:37 **EVALUATING THE FEASIBILITY OF FLOOD-ILLUMINATED ADAPTIVE OPTICS IN THE CLINICAL SETTING WITH PATIENTS WITH INHERITED RETINAL DEGENERATION**
MARK PENNESI, Michael Gale, Shu Feng, Hope Titus, Travis Smith

16:30 – 18:30 **OI02 – Innovations in Imaging Animal Models of Disease**

Moderator: Hao Zhang

Marina

- 16:38 **IMAGING RETINAL OXYGEN METABOLISM IN RODENT EYES**
JI YI, Wenzhong Liu, Siyu Chen, Robert Linsenmeier, Hao Zhang
- 17:06 **RETINAL VASCULAR OXYGEN TENSION IMAGING IN RODENT MODELS OF RETINOPATHY**
MAHNAZ SHAHIDI
- 17:34 **INTERACTION OF OCULAR TISSUES WITH ACOUSTIC RADIATION FORCE**
RONALD SILVERMAN, Raksha Urs, Harriet Lloyd
- 18:02 **BRILLOUIN MICROSCOPY FOR CORNEAL IMAGING**
SEOK-HYUN (ANDY) YUN

RPE Biology and Pathology

08:00 – 10:00 **RP01 – RPE Replacement and Tissue Engineering**

Moderator: Boris Stanzel

Bayview A

- 08:05 **TISSUE ENGINEERING CAN PAVE THE WAY FOR RPE FROM THE LAB INTO SUBRETINAL SPACE**
BORIS STANZEL
- 08:28 **STRATEGIES FOR CELLULAR THERAPY TO TREAT RETINAL DEGENERATION**
DENNIS CLEGG, Sherry Hikita, Qirui Hu, Linc.olin Johnson, Roxanne Croze, Tracy Clevenger, Lyndsay Leach, Britney Pennington

- 08:51 **CELL CARRIER SUBSTRATES FOR RPE REPLACEMENT**
CARL SHERIDAN, Victoria Kearns, Elies Alias, Rachel Williams
- 09:14 **DEVELOPMENT OF A RPE / CHOROIDAL STROMAL SUBSTITUTE MODEL USING THE SELF-ASSEMBLY APPROACH OF TISSUE ENGINEERING**
STEPHANIE PROULX, Olivier Rochette-Drouin, Véronique Beaulieu-Leclerc, Solange Landreville
- 09:37 **SURGICAL ASPECTS OF STEM CELL-DERIVED RETINAL PIGMENT TRANSPLANTATION IN ANIMALS**
MICHAEL KOSS

13:00 – 15:00 **RP02 – Phagosomes, Endosomes, and Lipofuscin in the RPE**

Seacliff C

Moderators: David Williams, Aparna Lakkaraju

- 13:05 **REDUCE, REUSE, RECYCLE: MECHANISMS OF CELLULAR CLEARANCE IN THE RPE**
APARNA LAKKARAJU, Kimberly Toops, Li Xuan Tan
- 13:30 **THE CONTRIBUTION OF MELANOREGULIN TO MICROTUBULE-ASSOCIATED PROTEIN 1 LIGHT CHAIN 3 (LC3) ASSOCIATED PHAGOCYTOSIS BY THE RETINAL PIGMENT EPITHELIUM**
KATHLEEN BOESZE-BATTAGLIA, Laura Frost, Vanda Lopes, Ignacio Rodriguez, David Williams
- 13:55 **ROLE OF KINESIN-1 IN THE TRAFFICKING OF PHAGOSOMES AND ENDOSOMES IN THE RPE**
MEI JIANG, Tanja Diemer, Vanda Lopes, David Williams, Agrani Rump
- 14:15 **REMOVAL OF RPE LIPOFUSCIN BY CYCLODEXTRINS**
ENRIQUE RODRIQUEZ-BOULAN, Marcelo Nociari, Guillermo Lehmann, Andres Perez Bay, Roxana Radu, Ryan Schreiner, David Warren, Jufang Shan, Segolene De Beaumais, Mickael Menand, Mathieu Sollogoub, Frederick Maxfield
- 14:25 **PHAGOSOME MATURATION IN RETINAL PIGMENT EPITHELIAL CELLS IN HEALTH AND RETINAL DEGENERATIVE DISEASE**
CLARE FUTTER, Silene Wavre-Shapton, Ingrid Meschede, Tanya Tolmachova, Thomas Burgoyne, Emily Eden, Hannah Mitchison, Miguel Seabra
- 14:45 **ESSENTIAL ROLE OF THE SODIUM / HYDROGEN EXCHANGER 8 IN THE RETINA**
CHUN-HONG XIA, Felicia Tang, Xiaohua Gong

16:30 – 18:30 **RP03 – Very Long Chain Fatty Acids in Ocular Health and Disease**

Seacliff C

Moderators: Julia Busik, Robert Eugene Anderson

- 16:35 **INTER-RELATIONSHIPS OF HUMAN RETINAL VLC-PUFAS WITH BIOMARKERS OF DIETARY LIPID INTAKE**
PAUL BERNSTEIN, Aruna Gorusupudi, Aihua Liu
- 16:55 **VERY LONG CHAIN FATTY ACIDS: MORE THAN NEEDS THE EYE**
MARTIN-PAUL AGBAGA, Blake Hopiavuori, Shelby Wilkinson, Feng Li, Nawajes Mandal, Michael Elliott, Richard Brush, Radha Ayyagari, Robert Anderson
- 17:15 **EXPRESSION OF ELOVL4 IN THE MEIBOMIAN GLANDS OF MICE EXPLAINS THE PRESENCE OF EXTREMELY LONG CHAIN LIPIDS IN MEIBUM**
IGOR BUTOVICH, Jadwiga Wojtowicz, Anne McMahon
- 17:35 **STARGARDT-3 MACULAR DEGENERATION: DEFECTIVE PROCESSING OF PHOTORECEPTOR OUTER SEGMENTS BY RPE CELLS**
JULIAN ESTEVE-RUDD, Tanja Diemer, Zhichun Jiang, Roni Hazim, Kang Zhang, Roxana Radu, David Williams

- 17:55 VERY LONG CHAIN SPHINGOLIPIDS AND VASCULAR PERMEABILITY IN DIABETIC RETINOPATHY – THE “SKIN EFFECT” IN THE EYE
JULIA BUSIK, Todd Lydic, Vince Chiodo, Sanford Boye, William Hauswirth, Gavin Reid
- 18:10 CREL IS AN INTRACELLULAR MESSENGER OF THE ESSENTIAL DOCOSAHEXAENOIC ACID-DERIVED MEDIATOR NEUROPROTECTIN D1 IN RPE CELL SURVIVAL
NICOLAS BAZAN, Aram Asatryan, Jorgelina Calandria

Retinal Neuroscience and Development

08:00 – 10:00 **RN01 – Gene Regulation and Protein Modulation in Retinal Development**

Seacliff B

Moderator: Takahisha Furukawa

- 08:06 CONTROL OF NOTCH SIGNALING, PROGENITOR COMPETENCE AND GLIAL AND AMACRINE CELL DIFFERENTIATION BY LHX2
SETH BLACKSHAW, Jimmy de Melo, Cristina Zibetti
- 08:30 NUCLEAR RECEPTORS AND TRANSCRIPTIONAL CONTROL OF RETINAL NEUROGENESIS
DOUGLAS FORREST, Hong Liu, Yulong Fu, Anand Swaroop, Soo-young Kim, Lily Ng
- 08:54 ONECUT1 AND ONECUT2 REDUNDANTLY REGULATE EARLY RETINAL CELL FATES IN DEVELOPMENT
XIUQIAN MU, Darshan Sapkota, Hemabindu Chintala, Fuguo Wu, Steven Fliesler, Zihua Hu
- 09:18 FOXN4 AND FOXN4-REGULATED DLL4-NOTCH SIGNALING IN RETINAL CELL DEVELOPMENT
MENGQING XIANG, Huijun Luo, Mohammed Islam, Haisong Jiang, Min Zou, Li Cai, David Shima
- 09:42 A ROLE OF THE HOMEOPROTEIN TRANSCRIPTION FACTOR RAX IN POSTNATAL PHOTORECEPTOR DEVELOPMENT
TAKAHISA FURUKAWA

13:00 – 15:00 **RN02 – Development of Retinal Circuitry and Synapses**

Seacliff B

Moderator: Ning Tian

- 13:05 SYNAPTIC CIRCUITRY MEDIATING ROD AND CONE SIGNAL IN THE MAMMALIAN RETINA
SAM WU
- 13:30 MATURATION OF SYNAPTIC CIRCUITRY UNDERLYING MOTION DETECTION
WEI WEI
- 13:55 MECHANISM OF DIFFERENTIAL ADHESION MEDIATED CIRCUIT FORMATION AND SPACING
PETER FUERST
- 14:20 TRANSCRIPTIONAL CONTROL OF RETINAL AMACRINE SUBTYPE DEVELOPMENT
LIN GAN
- 14:45 PATTERNS OF GLUTAMATE RESPONSE IN OFF BIPOLAR CELLS OF RAT RETINA
ALEX VIELMA, Marco Fuenzalida, Oliver Schmachtenberg

16:30 – 18:30 RN03 – Mechanisms of Neuroprotection

Seacliff B

Moderator: Xian-Jie Yang

- 16:35 PROLONGING CONE SURVIVAL IN RETINITIS PIGMENTOSA
CLAUDIO PUNZO
- 17:00 REPROGRAMMING PHOTORECEPTORS
JOSEPH CORBO
- 17:25 CYTOKINE AND DRUG THERAPIES THAT TARGET MITOCHONDRIA AND ENERGY PRODUCTION TO PREVENT DEATH OF PHOTORECEPTORS AND RPE
JOHN ASH, Lei Xu
- 17:50 MECHANISMS OF CYTOKINE-MEDIATED NEUROPROTECTION
XIAN-JIE YANG, Kun-Do Rhee, Kevin Chao, Steve Nusinowitz, Dean Bok
- 18:15 MYOCILIN-DEFICIENT MICE ARE PROTECTED FROM NEURONAL DAMAGE IN THE RETINA
MARCUS KOCH, Bernd Rosenhammer, Sebastian Koschade, Barbara Braunger, Cornelia Volz, Herbert Jaegle, Ernst Tamm

Retinal Cell Biology

08:00 – 10:00 RC01 – Role of Retinal Clocks in Eye Disorders

Bayview B

Moderators: Gianluca Tosini, Mario Guido

- 08:00 CIRCADIAN REGULATION OF RETINAL FUNCTIONS AND PATHOPHYSIOLOGY
GIANLUCA TOSINI
- 08:20 RETINAL MÜLLER CELLS ARE CLOCK CELLS AND CLOCK GENES IMPACT RETINAL NEOVASCULARIZATION
DOUGLAS MCMAHON, Lili Xu, John Penn
- 08:40 RETINAL GANGLION CELLS IN BIRDS: DAY-TIMERS AND NON-VISUAL PHOTORECEPTORS
MARIO GUIDO, Diego Valdez, Paula Nieto, Maria Contin, Daniela Verra, Nicolas Diaz, Eduardo Garbarino-Pico
- 09:00 THE ROLE OF MELANOPSIN IN THE MAMMALIAN RETINAL CLOCK
OURIA DKHISSI-BENYAHYA
- 09:20 MULTIPLE PATHWAYS FOR THE CIRCADIAN REGULATION OF THE RETINAL PHAGOCYTOSIS RECEPTOR MERTK
CELIA PARINOT, Jonathan Chatagnon, Emeline Nandrot
- 09:40 CIRCADIAN REGULATION OF PHOTOPIC VISUAL FUNCTION: DIFFERENTIAL ROLES OF NPAS2 AND CLOCK
MICHAEL IUUVONE, Christopher Hwang

13:00 – 15:00 RC02 – Retinal TRP Channels and Visual Function

Bayview B

Moderator: David Krizaj

- 13:04 TRPV1 MODULATES VISUAL RESPONSES IN THE RETINA
MAUREEN MCCALL, Jennifer Noel

13:33 **MECHANISMS GATING TRPM1 IN RETINAL ON-BIPOLAR CELLS**
RONALD GREGG, Thomas Ray, Kathryn Heath, Nazarul Hasan, Jennifer Noel, Ivy Samuels, Kiril Martemyanov,
Maureen McCall, Neal Peachey

14:02 **ACTIVATION AND MODULATION OF THE TRPM1 CHANNEL IN ON-BIPOLAR CELLS**
NOGA VARDI, Ying Xu

14:31 **TRPV CHANNELS REGULATE NEURONAL AND GLIAL PHYSIOLOGY IN THE MAMMALIAN RETINA**
DANIEL RYSKAMP, David Krizaj

16:30 – 18:30 RC03 – Retinal Calcium-Binding Proteins: Structure, Function, and Role in Diseases Bayview B
Moderators: Alexander Dizhoor, James Ames

16:35 **GUANYLYL CYCLASE-ACTIVATING PROTEINS IN SIGNAL TRANSDUCTION AND RETINAL DISEASES: DO THEY COMPETE FOR THE SAME TARGET ENZYME OR ACT SYNERGISTICALLY?**
ALEXANDER DIZHOOR, Igor Peshenko, Elena Olshevskaya

16:50 **STRUCTURAL DIVERSITY OF NEURONAL CALCIUM SENSOR PROTEINS AND INSIGHTS FOR ACTIVATION OF RETINAL GUANYLYL CYCLASE BY GCAP1**
JAMES AMES

17:15 **IDENTIFICATION OF THE INTERFACE FOR THE TARGET ENZYME ACTIVATION IN GCAP1**
IGOR PESHENKO

17:40 **DIVERSITY OF NEURONAL CALCIUM SENSOR PROTEINS IN ZEBRAFISH RODS AND CONES**
KARL-WILHELM KOCH, Alexander Scholten, Christian Kramer, Jingjing Zang, Stephan Neuhauss

18:05 **EML1 (CNG-MODULIN) MODULATES CONE CGMP-GATED CHANNEL AND CONTROLS LIGHT SENSITIVITY IN FISH RETINAL CONE PHOTORECEPTORS**
TATIANA REBRIK, Nomi Tserentsoodol, Juan Korenbrot

Tuesday, 22 July 2014

Lens

08:00 – 10:00 LE04 – Communication in the Eye: Channels and Transporters

Seacliff A

Moderators: Barbara Kloeckener-Gruissem, Viviana Berthoud

- 08:00 A CX46 FRAME-SHIFT MUTANT, CX46FS380, CAUSES PROGRESSIVE CATARACTS IN THE MOUSE LENS
VIVIANA BERTHOUD, Peter Minogue, Helena Yu, Joe Snabb, Eric Beyer
- 08:17 MECHANISMS REGULATING INTRACELLULAR HYDROSTATIC PRESSURE AT THE SURFACE OF THE MOUSE LENS
RICHARD MATHIAS, Junyuan Gao, Xiurong Sun, Thomas White, Nicholas Delamere
- 08:34 THE REGULATION OF CATION CHLORIDE COTRANSPORTERS IN THE LENS
PAUL DONALDSON, Irene Vorontsova, Julie Lim
- 08:51 AQUAPORIN ZERO REGULATION AND ROLES IN LENS DEVELOPMENT AND HOMEOSTASIS
JAMES HALL, Daniel Clemens, Karine Németh-Cahalan, Thomas Schilling
- 09:08 RELATIONSHIP BETWEEN STRUCTURE OF THE MONOCARBOXYLATE TRANSPORTER MCT12 AND ITS FUNCTION IN CATARACT FORMATION AND CREATINE TRANSPORT
BARBARA KLOECKENER-GRUISSEM, Michel Bielecki, Pooja Gangras, Sabrina Steiner, François Verrey, Simone Camargo, Wolfgang Berger
- 09:25 METABOLIC COUPLING BETWEEN THE RETINA PIGMENT EPITHELIUM AND THE NEURAL RETINA: THE RPE PRODUCES BETA-HYDROXYBUTYRATE TO SUPPORT RETINAL METABOLISM
NANCY PHILP, Jeffrey Adijanto
- 09:42 HUMAN OCULAR TRANSPORTERS: TRANSPORTER MEDIATED INTRAOCULAR DRUG DELIVERY
SUNIL VOOTURI

13:00 – 15:00 LE05 – Cataract Genetics and Genomics

Seacliff A

Moderators: Alan Shiels, Jochen Graw

- 13:00 CATARACT GENE DISCOVERY VIA NEXT-GENERATION SEQUENCING
ALAN SHIELS, Thomas Bennett, Donna Mackay
- 13:20 WHOLE EXOME SEQUENCING OF PEDIATRIC CATARACT: LESSONS LEARNED, NOVEL CANDIDATES AND FUTURE DIRECTIONS
ELENA SEMINA, Linda Reis
- 13:40 CHARACTERIZATION OF A DNA-REPAIR GENE (*ERCC2*) IN A RECESSIVE CATARACT IN THE MOUSE - UNEXPECTED FINDING BY EXOME SEQUENCING
JOCHEN GRAW, Sarah Kunze, Helmut Fuchs, Matthias Klaften, Sibylle Sabrautzki, Martin Hrabé de Angelis, Claudia Dalke
- 14:00 POSITIONAL CLONING OF THE GENE FOR HEREDITARY CATARACT IN THE NAKANO MOUSE REVEALS INVOLVEMENT OF THE HEME BIOSYNTHESIS PATHWAY IN LENS PHYSIOLOGY
MASAYUKI MORI, Shigeru Taketani, Keiichi Higuchi, Hiroshi Hiai
- 14:20 MOLECULAR ANALYSIS OF CONGENITAL CATARACT CAUSING MUTATIONS IN THE *EPHA2* GENE
ALPANA DAVE, Hidetoshi Tanioka, Takaharu Mochizuki, Takashi Yamanouchi, Osamu Katsuta, Kouichi Kawazu

14:40 A PHARMACOLOGICAL CHAPERONE FOR A CHAPERONE PROTEIN: REVERSAL OF CATARACTS CAUSED BY A MUTATION IN ALPHAB-CRYSTALLIN
LEAH MAKLEY, Usha Andley, Jason Gestwicki

16:30 – 18:30 **LE06 – Death and Differentiation: Novel Pathways in the Lens** Seacliff A
Moderators: Sue Menko, Marc Kantorow

16:30 SUPPRESSION OF JNK-MTOR SIGNALING PATHWAY INDUCES PREMATURE FORMATION OF THE ORGANELLE FREE ZONE BY AUTOPHAGY DURING LENS FIBER CELL DIFFERENTIATION
SUE MENKO, Subhasree Basu, Suren Rajakaruna

16:50 INTEGRIN α V β 5-MEDIATED PHAGOCYTOSIS BY LENS EPITHELIAL CELLS INCREASES CELL SURVIVAL UNDER APOPTOTIC CONDITIONS
MARC KANTOROW, Daniel Chauss, Lisa Brennan

17:10 AUTOPHAGY AND THE UNFOLDED PROTEIN RESPONSE IN MOUSE MODELS OF HEREDITARY CATARACTS
USHA ANDLEY

17:30 INTEGRATED PROTEOLYTIC CONTROL IN LENS AND RETINA BY UBIQUITIN, AUTOPHAGIC, AND CALPAIN SYSTEMS
ALLEN TAYLOR, Ke Liu, Tomoaki Uchiki, Fu Shang, Min-Lee Chang, Richard Mathias, Sheldon Rowan, Hideko Kasahara

17:50 CHROMATIN STRUCTURE AND DYNAMICS AND THEIR ROLES IN LENS FIBER CELL DENUCLEATION
ALES CVEKL, Shuying He, Saima Limi, Qing Xie, Tomas Stopka, Arthur Skoultchi

18:10 REPAIR CELL ACTIVATION IN RESPONSE TO LENS DYSMORPHOGENESIS CAN INDUCE PROPERTIES ASSOCIATED WITH FIBROSIS
CAITLIN LOGAN, Liping Zhang, Sue Menko

Glaucoma

08:00 – 10:00 **GL04 – Glaucoma Genetics** Grand Ballroom
Moderators: Mary Wirtz, Francesca Pasutto, David Mackey

08:00 TRANSGENIC ANIMAL- AND STEM CELL-BASED APPROACHES TO STUDY NORMAL TENSION GLAUCOMA
JOHN FINGERT, Michael Anderson, Robert Mullins, Budd Tucker

08:17 GENOME-WIDE ASSOCIATION STUDIES OF OPHTHALMOLOGY
DAVID MACKEY

08:34 PRIMARY OPEN ANGLE GLAUCOMA: GENOME-WIDE ASSOCIATION STUDIES
JANEY WIGGS

08:51 INTERLEUKIN-20 RECEPTOR EXPRESSION IN TRABECULAR MESHWORK AND ITS IMPLICATIONS IN GLAUCOMA
KATE KELLER

09:08 EXOME SEQUENCING REVEALED AN ASSOCIATION OF PRIMARY GLAUCOMA WITH MUTATIONS IN GENES RELATED TO ANTERIOR SEGMENT DYSGENESIS
QINGJIONG ZHANG, Xiaobo Huang, Xueshan Xiao

- 09:25 IDENTIFICATION AND IN VIVO VALIDATION OF DELETIONS OF REGULATORY ELEMENTS IN PITX2 AS CAUSATIVE FOR DEVELOPMENTAL GLAUCOMA
MEREDITH PROTAS, Tim Footz, Scott Baraban, Michael Walter, Ordan Lehmann, Douglas Gould
- 09:42 GENE AND ENVIRONMENT INTERACTION IN THE ONSET AND PROGRESSION OF MYOPIA: GUANGZHOU TWIN EYE STUDY
MINGGUANG HE

13:00 – 15:00 GL05 – Animal Models of Glaucoma

Grand Ballroom

Moderators: John Morrison, Iok-Hou Pang

- 13:00 DEFINING AN AUTOIMMUNE MECHANISM OF GLAUCOMA IN A MICROBEAD-INDUCED DISEASE MODEL
DONG CHEN
- 13:20 AN INDUCIBLE MOUSE MODEL OF PIGMENT DISPERSION SYNDROME
MICHAEL ANDERSON, Adam Hedberg-Buenz
- 13:40 INVESTIGATION OF PATHOGENIC PATHWAYS OF GLAUCOMA IN AN INDUCIBLE MOUSE MODEL SYSTEM
COLLEEN MCDOWELL, Abbot Clark
- 14:00 LONGITUDINAL CHARACTERIZATION OF LOW TENSION GLAUCOMA IN MICROFIBRIL DEFICIENT MICE
RACHEL KUCHTEY, Monique McCallister, Jessica Kunkel, John Scichilone, John Kuchtey
- 14:20 CONTINUOUS MEASUREMENT AND CONTROL OF INTRAOCULAR PRESSURE IN RATS
CHRISTOPHER PASSAGLIA, Simon Bello, Xiaolan Tang, Sharad Malavade
- 14:40 GENETIC VARIANTS OF GENE SIX6 INCREASE POAG RISK, REDUCE RNFL IN POAG CASES, AND REDUCE OPTIC NERVE VOLUME IN THE ZEBRAFISH MODEL
R RAND ALLINGHAM, Megan Ulmer, Yangfan Liu, Edwin Oh, Nicholas Katsanis, Yutao Liu, Louis Pasquale, Janey Wiggs, Allison Ashley-Koch, Shane Havens, Michael Hauser

16:30 – 18:30 GL06 – Biomechanics in Glaucoma: Trabecular Meshwork and Optic Nerve Head

Grand Ballroom

Moderators: Darryl Overby, J. Crawford Downs

- 16:30 OCULAR BIOMECHANICS IN THE ANTERIOR AND POSTERIOR SEGMENTS
J. CRAWFORD DOWNS, Darryl Overby
- 16:36 DEXAMETHASONE MODULATES TRABECULAR MESHWORK CELL MECHANICS
VIJAY KRISHNA RAGHUNATHAN, Joshua Morgan, Christopher Murphy, Paul Russell
- 16:55 ELEVATED PRESSURE INDUCES ECM GENE EXPRESSION CHANGES IN LOW AND HIGH FLOW REGIONS OF THE HUMAN TRABECULAR MESHWORK
JANICE VRANKA, Ted Acott
- 17:14 THE ROLE OF BIOMECHANICAL INSULT OF THE OPTIC NERVE HEAD IN THE DEVELOPMENT OF GLAUCOMA
JOHN FLANAGAN, Jeremy Sivak
- 17:33 AGE AND RACE SIGNIFICANTLY IMPACT THE IOP-INDUCED MECHANICAL ENERGY ABSORPTION IN THE POSTERIOR HUMAN SCLERA
MASSIMO FAZIO, Jeff Morris, Rafael Grytz, Luigi Bruno, Christopher Girkin, J Crawford Downs

17:52 **USE OF MICROARCHITECTURAL INFORMATION TO QUANTIFY LAMINA
CRIBROSA BIOMECHANICS**
C. ROSS ETHIER, Ian Campbell, Baptiste Coudrillier

18:11 **THE IMPLICATIONS OF VARIATION IN THREE-DIMENSIONAL MORPHOMETRY OF THE HUMAN
LAMINA CRIBROSA ON THE PATHOGENESIS OF GLAUCOMA**
CHRISTOPHER GIRKIN, J. Crawford Downs

Cornea and Ocular Surface

08:00 – 10:00 C004 – Corneal Stromal and Endothelial Biology
Moderators: Shigeru Kinoshita, Winston Kao

Seacliff D

08:00 **THE BIOLOGICAL CHARACTERISTICS OF HUMAN CORNEAL ENDOTHELIAL CELLS**
SHIGERU KINOSHITA

08:18 **OXIDATIVE DNA DAMAGE IN FUCHS ENDOTHELIAL CORNEAL DYSTROPHY**
ADNA HALILOVIC, Ula Jurkunas, Thore Schmedt, Cecily Hamill

08:36 **NOVEL STRATEGIES FOR ENGINEERING HUMAN CORNEAL ENDOTHELIUM BY HC-HA / PTX3
REGENERATIVE MATRIX FROM AMNIOTIC MEMBRANE AND P120 CATENIN-KAISO SIGNALING**
SCHEFFER TSENG, Ying-Tieng Zhu

08:54 **ROLES OF TRP CHANNELS IN SMAD SIGNAL AND MYOFIBROBLAST FORMATION IN
STROMAL REPAIR**
SHIZUYA SAIKA, Peter Reinach, Yuka Okada

09:12 **INTERRUPTION OF WNT / β -CATENIN SIGNALING AXIS IN KERATOCYTES CAUSES PRECOCIOUS
CORNEAL EPITHELIUM STRATIFICATION VIA BMP4**
WINSTON KAO, Yujin Zhang, Lung-Kun Yeh, Chia-Yang Liu

09:30 **INVOLVEMENT OF TRANSFORMING GROWTH FACTOR BETA IN EXTRACELLULAR MATRIX
DEPOSITION AND ENDOPLASMIC RETICULUM STRESS IN FUCHS' CORNEAL DYSTROPHY**
NAOKI OKUMURA, Ryuki Minamiyama, EunDuck Kay, Leona Ho, Satoshi Kawasaki, Robert Young,
Andrew Quantock, Ursula Schlötzer-Schrehardt, Friedrich Kruse, Shigeru Kinoshita, Noriko Koizumi

09:40 **CORNEAL STROMAL SCARRING: TARGETING THE DEUBIQUITINASE USP10 PREVENTS INTEGRIN-
MEDIATED FIBROSIS**
AUDREY BERNSTEIN, Liana Tedesco, Lingyan Wang, Stephanie Gillespie

13:00 – 15:00 C005 – Corneal Development, Differentiation, and Genetics
Moderators: David Beebe, M. Elizabeth Fini

Seacliff D

13:00 **INTERACTION BETWEEN HEDGEHOG SIGNALING, RETINOIC ACID AND PAX6 DURING CORNEAL
REGENERATION**
J. MARTIN COLLINSON, Romana Kucerova, Natalie Dora, John West

13:20 **FGF AND WNT SIGNALING AND THE HOMEBOX TRANSCRIPTION FACTOR, OTX1, PATTERN THE
OCULAR SURFACE EPITHELIA AND REGULATE THE EXPRESSION OF SIX2 AND SIX3**
JIE HUANG, Ying Liu, Ziyang Chen

13:40 **FUCHS ENDOTHELIAL CORNEAL DYSTROPHY: GENETICS, PATHOGENESIS, AND POTENTIAL NON-
SURGICAL TREATMENT**
ALBERT JUN

13:55	GENETICS OF CORNEAL THICKNESS XIAOYI GAO
14:10	NOVEL MEMBRANE-TETHERED MUCINS AT THE OCULAR SURFACE AND IN THE AQUEOUS OUTFLOW PATHWAY M. ELIZABETH FINI, Shinwu Jeong, Minako Hijikata, Keicho Naoto, Haiyan Gong, Pablo Argüeso
14:25	MOUSE GENOMIC LOCI MODULATING CENTRAL CORNEAL THICKNESS ELDON GEISERT, Xiangdi Wang, Steven Hart, Michael Hauser, Louis Pasquale, Janey Wiggs
14:35	USING MOUSE GENETICS TO IDENTIFY QTL ASSOCIATED WITH CENTRAL CORNEAL THICKNESS DEMELZA KOEHN, Michael Anderson

16:30 – 18:30 **CO06 – Corneal Innervation: Lesson from the Basic Science**

Seacliff D

Moderators: Juana Gallar, Haydee Bazan

16:30	IN VIVO VISUALISATION OF MURINE CORNEAL NERVE FIBRE REGENERATION IN RESPONSE TO CILIARY NEUROTROPHIC FACTOR OLIVER STACHS, Maria Reichard, Marina Hovakimyan, Rudolf Guthoff
16:50	THE REGENERATIVE AND INFLAMMATORY BIOACTIVITY OF PIGMENT EPITHELIAL DERIVED FACTOR (PEDF) AND DOCOSAHEXAENOIC ACID (DHA) AFTER CORNEAL NERVE DAMAGE HAYDEE BAZAN
17:10	MYELOID CELL-MEDIATED NERVE REGENERATION – LESSONS LEARNED AND TRANSLATIONAL ASPECTS SANDEEP JAIN
17:25	FROM THE CORNEA TO THE BRAIN AND BACK: A NOVEL ROLE FOR CORNEAL NERVES GIULIO FERRARI
17:40	FAILED REINNERVATION AT SITES OF CORNEAL EROSIONS MARY ANN STEPP, Sonali Pal-Ghosh, Gauri Tadvalkar, Ahdreah Pajooohesh-Ganji
17:55	CORNEAL NERVE ACTIVITY AND OCULAR SENSATIONS UNDER PATHOLOGICAL CONDITIONS JUANA GALLAR, M. Carmen Acosta
18:10	CORNEAL SENSITIVITY LOSS IN PTERYGIUM PATIENTS: A COMPARATIVE ANALYSIS BETWEEN AFFECTED AND UNAFFECTED ZONE PAMELA CAMPOS FIGUEROA, Gemma Julio Morán, Moufak Asaad, Pere Pujol Vives

Ocular Immunology

08:00 – 10:00 **IM03 – New Insights into the Immunology of Ocular Tumors**

Golden Gate

Moderator: Ludwig Heindl

08:10	MACROPHAGE INFILTRATION IN UVEAL MELANOMA: ENVIRONMENT OR GENETICS? MARTINE JAGER, Inge Bronkhorst, Tina Jehs, Eveline Dijkgraaf, Pieter Van Der Velden, Gregorius Luyten, Sjoerd Van Der Burg
08:35	TUMOR-ASSOCIATED LYMPHANGIOGENESIS AND ITS INHIBITION IN OCULAR SURFACE MALIGNANCIES LUDWIG HEINDL

08:50	AUTOCRINE IMPACT OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) A ON PRIMARY AND METASTATIC UVEAL MELANOMA CELLS KONRAD KOCH, Nasrin Refaian, Deniz Hos, Simona Schlereth, Jacobus Bosch, Claus Cursiefen, Ludwig Heindl
09:10	IMMUNOLOGICAL TREATMENTS FOR UVEAL MELANOMA PIERRE TRIOZZI
09:35	TOWARDS T CELL-BASED IMMUNOTHERAPY OF UVEAL MELANOMA JACOBUS BOSCH

Physiology and Pharmacology

08:00 – 10:00 **PH03 – Photoreceptor Physiology and Adaptation** Marina Moderator: Marie Burns

08:05	SENSITIVITY RECOVERY OF CONE PHOTORECEPTORS UNDER CONTINUOUS ILLUMINATION: DIFFERENCES IN FISH AND MONKEY RETINAS FIT BY A SINGLE SIMULATION MODEL JUAN KORENBROT, Jan Verweij, James Long, Julie Schnapf
08:28	MOLECULAR MECHANISMS THAT CONTROL RESPONSE KINETICS IN MOUSE RODS, SOME UNUSUAL SUSPECTS MICHAEL WOODRUFF, Ching-Kang Chen, Daniel Tranchina, Gordon Fain
08:51	MODULATION OF PHOTOTRANSDUCTION AND PHOTORECEPTOR CELL SURVIVAL IN KV2.1 POTASSIUM CHANNEL KNOCKOUT MICE SYED ABBAS, Christopher Kessler, Emily Levine, Hannah Bishop, James Trimmer, Marie Burns
09:14	CHARACTERIZATION OF DARK NOISE IN ROD PHOTORECEPTORS AND IMPLICATIONS FOR VISUAL THRESHOLD ALAPAKKAM SAMPATH, Johan Pahlberg
09:37	ADAPTIVE POTENTIATION IN ROD PHOTORECEPTORS TIMOTHY KRAFT, Alex McKeown

16:30 – 18:30 **PH04 – Defects in Ion Channel Function Causing Disease** Marina Moderator: Bikash Pattnaik

16:35	RESTORATION OF VISUAL FUNCTION TO BLIND MICE WITH A CHEMICAL PHOTOSWITCH TARGETED TO DEGENERATED RETINA IVAN TOCHITSKY, Aleksandra Polosukhina, Vadim Degtyar, Nicholas Gallerani, Caleb Smith, Aaron Friedman, Russell Van Gelder, Dirk Trauner, Daniela Kaufer, Richard Kramer
17:03	DISRUPTION IN KIR7.1 CHANNEL LEADS TO BLINDNESS BIKASH PATTNAIK, Pawan Shahi, Xinying Liu, Nathan York, Simran Brar, Wenxiang Liu, De-Ann Pillers
17:31	ROLE OF MECHANOSENSITIVE TRP CHANNELS IN RETINAL PATHOLOGY DANIEL RYSKAMP, Amber Frye, Peter Barabas, Tünde Molnár, Andrew Jo, Erik Soderborg, David Krizaj
17:59	VOLTAGE DEPENDENT CALCIUM CHANNELS IN STEM CELL DERIVED RETINAL PIGMENT EPITHELIUM SOILE NYMARK, Iina Vainio, Kati Juuti-Uusitalo, Heli Skottman, Jari Hyttinen

Ocular Imaging

08:00 – 10:00 **OI03 – Imaging Structure and Function in Glaucoma**

Seacliff C

Moderator: Jason Porter

- 08:00 **IN VIVO IMAGING OF THE 3D LAMINA CRIBROSA MICROARCHITECTURE IN NORMAL AND GLAUCOMATOUS HUMAN EYES**
GADI WOLLSTEIN, Bo Wang, Zach Nadler, Hiroshi Ishikawa, Richard Bilonick, Larry Kagemann, Ian Sigal, Joel Schuman
- 08:25 **HIGH-RESOLUTION IN VIVO CHARACTERIZATION OF LAMINA CRIBROSA MICROARCHITECTURE AND OPTIC NERVE HEAD CHANGES IN EARLY GLAUCOMA**
JASON PORTER, Kevin Ivers, Nripun Sredar, Nimesh Patel, Lakshmi Rajagopalan, Hope Queener, Ronald Harwerth, George Zouridakis
- 08:50 **ASSESSING FOCAL RETINAL GANGLION CELL FUNCTION IN GLAUCOMA USING THE ELECTRORETINOGRAM**
SURESH VISWANATHAN, Lakshmi Rajagopalan, Anthony VanAlstine, Nimesh Patel, Ronald Harwerth, Laura Frishman
- 09:15 **ASSESSING NEURONAL DAMAGE CAUSED BY GLAUCOMA**
RONALD HARWERTH, Nimesh Patel
- 09:40 **QUANTUM DOTS TRAVEL FROM THE ANTERIOR CHAMBER OF THE EYE INTO THE OPTIC NERVE AND THIS IS ENHANCED BY LATANOPROST**
EMILY MATHIEU, Farhana Islam, Neeru Gupta, Alex Tam, Yeni Yucel

16:30 – 18:30 **OI04 – New Approaches to Imaging Retinal and Choroidal Vasculature**

Seacliff B

Moderator: Steve Burns

- 16:30 **EFFECT OF ANTI-VEGF THERAPY ON CHOROIDAL THICKNESS IN DIABETIC MACULAR EDEMA**
GLENN YIU, Varsha Manjunath, Stephanie Chiu, Sina Farsiu, Tamer Mahmoud
- 17:00 **PROGRESS ON OCT BASED IMAGING OF CHORIOCAPILLARIS IN THE LIVING HUMAN EYE**
ROBERT ZAWADZKI
- 17:30 **PHOTOACOUSTIC VASCULAR IMAGING IN THE EYE**
HAO ZHANG, Wenzhong Liu
- 18:00 **VASCULAR IMAGING IN DIABETIC RETINOPATHY**
ANN ELSNER, Joel Papay, Stephen Burns, Jason Green, Mastour Alhamami, Karthikeyan Baskaran, Brett King, Thomas Gast, Dean VanNasdale, Matthew Muller

RPE Biology and Pathology

08:00 – 10:00 **RP04 – RPE and the Complement System**

Bayview A

Moderators: Stephen Moss, Catherine Bowes-Rickman

- 08:06 **INTERACTIONS OF COMPLEMENT FACTOR-H AND FACTOR H LIKE PROTEIN-1 WITH MACULAR TISSUE**
PAUL BISHOP, Simon Clark, Alexander Langford-Smith, Anthony Day

08:27	TARGETED DELETION OF COMPLEMENT FACTOR H IN THE MOUSE RPE STEPHEN MOSS
08:48	LESSONS LEARNED FROM MURINE MODELS OF COMPLEMENT DYSREGULATION CATHERINE BOWES RICKMAN, Una Kelly, Michael Landowski, Christopher Toomey, J. Mikael Klingeborn, Jindong Ding
09:09	COMPLEMENT REGULATION AND DYSREGULATION AS A FUNCTION OF GENOTYPE IN CULTURED HUMAN RETINAL PIGMENT EPITHELIUM DEAN BOK, Jane Hu, Zhichun Jiang, Roxana Radu
09:30	HOMOZYGOUS RISK FOR ARMS2 AND CFH CORRELATES WITH INCREASED MTDNA DAMAGE: DEVELOPING PERSONALIZED MEDICINE TO TREAT AGE-RELATED MACULAR DEGENERATION DEBORAH FERRINGTON, Marcia Terluk, Mara Ebeling, Pabalu Karunadharma, Sandra Montezuma, Shari Atilano, Cristina Kenney
09:45	A CELL CULTURE MODEL FOR GEOGRAPHIC ATROPHY DAVID FOREST, Lincoln Johnson

13:00 – 15:00 **RP05 – Choroid in Health and AMD**

Seacliff C

Moderators: Gerard Luty, Robert Mullins

13:05	INSULT BY COMPLEMENT: CHOROIDAL ENDOTHELIAL RESPONSE TO MAC DEPOSITION S. SCOTT WHITMORE, Shemin Zeng, Budd Tucker, Edwin Stone, Todd Scheetz, Robert Mullins
13:28	PERIVASCULAR MURAL CELLS AND DENDRITIFORM MYELOID CELLS IN THE MOUSE CHOROID – MORPHOLOGY, DISTRIBUTION, AND INTERACTIONS WAI WONG, Anil Kumar, Audree Condren
13:51	CD68+ AND CD163+ MACROPHAGES IN THE NORMAL HUMAN ADULT CHOROID SVETLANA CHEREPANOFF, Paul McMenamin, Enisa Hasic, Mark Gillies
14:14	INFLAMMATORY CELLS OF CHOROID IN AGING AND AMD GERARD LUTTY, Imran Bhutto, Johanna Seddon, D. S. McLeod
14:37	THE ROLE OF PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR (PPARβ/Δ) IN CHOROIDAL ANGIOGENESIS SARA SAVAGE, John Penn

16:30 – 18:30 **RP06 – Signaling Pathways and Organelle Dynamics**

Seacliff C

Moderators: Silvia Finnemann, Emeline Nandrot

16:36	REGULATION OF MERTK ACTIVITY DURING RPE PHAGOCYTOSIS BY EXTRACELLULAR PROTEINS EMELINE NANDROT, Jonathan Chatagnon, Célia Parinot
16:55	SIGNALING DOWNSTREAM OF MERTK IN RPE PHAGOCYTOSIS DEBRA THOMPSON, Shameka Shelby, Anna Ganos, Jingyu Yao, Kecia Feathers, Lin Jia, David Zacks
17:14	REGULATION OF AUTOPHAGY AND PHAGOCYTOSIS BY CRYBA1 IN RETINAL PIGMENT EPITHELIAL CELLS MALLIKA VALAPALA, J Samuel Zigler, Stacey Hose, Imran Bhutto, Rhonda Grebe, Eric Wawrousek, Gerard Luty, Debasish Sinha
17:33	ER STRESS SIGNALING IN REGULATION OF THE RPE BARRIER FUNCTION JACEY MA, Sarah Zhang

17:52 REGULATION OF RPE PHENOTYPE BY ANNEXIN A8 AND WNT SIGNALING
KATHARINA LUECK, John Greenwood, Stephen Moss

18:11 REGULATION OF LYSOSOMAL FUNCTION IN RPE CELLS
SILVIA FINNEMANN, Saumil Sethna, Michael Elliott

Retinal Neuroscience and Development

08:00 – 10:00 **RN04 – Glial Cells in Retina Regeneration and Repair**
Moderator: David Hyde

Seacliff B

08:05 MÜLLER GLIA-DERIVED PROGENITORS AND RETINAL REGENERATION
ANDY FISCHER, Donika Gallina

08:30 REPROGRAMMING ZEBRAFISH MÜLLER GLIA FOR RETINAL REPAIR
DANIEL GOLDMAN, Jin Wan, Xiao-Feng Zhao

08:55 EXPRESSING TNFA AND REPRESSING NOTCH SIGNALING ARE NECESSARY AND SUFFICIENT TO INDUCE MÜLLER GLIA DEDIFFERENTIATION AND PROLIFERATION IN THE ZEBRAFISH RETINA
DAVID HYDE, Clay Conner, Kristin Ackerman, Manuela Lahne, Joshua Hobgood

09:20 CHARACTERIZATION OF SONIC HEDGEHOG FUNCTION DURING MÜLLER GLIA-MEDIATED RETINAL REGENERATION IN ADULT ZEBRAFISH
RYAN THUMMEL, Jennifer Thomas

09:40 MOLECULAR AND STRUCTURAL ALTERATIONS OF MÜLLER GLIAL CELLS FROM THE "RD" RETINA
LUIS POLITI, Yanel Volonté, Lorena German, Victoria Simon, Nora Rotstein

13:00 – 15:00 **RN05 – RGC Development, Survival, and Axonal Guidance**
Moderator: David Feldheim

Seacliff B

13:05 DEVELOPMENT MECHANISMS OF VISUAL CIRCUIT FORMATION
DAVID FELDHEIM, Neal Sweeney

13:30 COMBINATORIAL CODES FOR RETINAL GANGLION CELL DEVELOPMENT
TUDOR BADEA

13:55 GENETIC AND VIRUS-BASED APPROACHES FOR PARSING THE CELL TYPES AND CIRCUITS MEDIATING DISCRETE VISUAL BEHAVIORS
ONKAR DHANDE

14:20 ASTROCYTE PHAGOCYTOSIS OF MYELIN DEBRIS DURING XENOPUS LAEVIS METAMORPHIC OPTIC NERVE SHORTENING
NICHOLAS MARSH-ARMSTRONG, Chung-ha Davis, Eric Bushong, Keun-Young Kim, Daniela Boassa, Mark Ellisman, Elizabeth Mills

14:40 TISSUE ENGINEERED CELL DELIVERY VEHICLE FOR TRANSPLANTATION OF RETINAL GANGLION CELLS
KARL KADOR, Shawn Grogan, Erik Dorthé, Monisha Malek, Darryl D'Lima, Jeffrey Goldberg

16:30 – 18:30 RN06 – Road to Cure I: Stem Cell and ES/iPS Cell Therapy

Bayview A

Moderator: Thomas Reh

- 16:30** REGENERATIVE MEDICAL APPROACHES FOR RETINAL DEGENERATIONS: A ROLE FOR MIRNAS
THOMAS REH, Anna LaTorre, Akina Hoshino, Lauren Hood
-
- 17:00** CLINICAL TRIALS OF EMBRYONIC STEM CELL-DERIVED RPE FOR RETINAL DEGENERATION
DAVID HINTON
-
- 17:30** GENERATING RETINAL CELLS FROM HUMAN ESCS AND IPSCS
DAVID GAMM, Michael Phillips, Elizabeth Capowski, Ruchira Singh, Lynda Wright
-
- 18:00** PHOTORECEPTOR TRANSPLANTATION: ROAD MAP FOR THERAPY
ROBIN ALI
-

Retinal Cell Biology

08:00 – 10:00 RC04 – Retinal Angiogenesis

Bayview B

Moderators: John Greenwood, Tailoi Chan-Ling

- 08:05** THE ROLE OF MYELOID-DERIVED CELLS IN INFLAMMATION-MEDIATED RETINAL ANGIOGENESIS
HEPING XU
-
- 08:25** NUCLEAR RECEPTOR RORALPHA CONTROL OF PATHOLOGIC RETINAL NEOVASCULARIZATION
JING CHEN
-
- 08:45** LRG1: A NEW THERAPEUTIC TARGET FOR THE TREATMENT OF RETINAL NEOVASCULAR COMPLICATIONS?
JOHN GREENWOOD, Xiaomeng Wang, Sabu Abraham, Sterenn Davis, Vineeta Tripathi, Stephen Moss
-
- 09:05** NETRIN-4 DOWNREGULATION MODULATES PATHOLOGIC OXYGEN-DRIVEN NEOVASCULARIZATION
ANTONIA JOUSSEN, Sabrina Klein, Yong Liang, Norbert Kociok, Sergej Skosyrski, Christina Nürnberg, Nadine Reichhart, William Brunken, Mats Paulsson, Manuel Koch, Olaf Strauss
-
- 09:20** AND THEN THERE WERE MILLIONS: NEW ANTIBODY THERAPY FOR ANGIOGENESIS
ANA BASTOS-CARVALHO, Valeria Tarallo, Benjamin Fowler, Bradley Gelfand, Sandro DeFalco, Jayakrishna Ambati, Sasha Bogdanovich, Younghee Kim, Takeshi Mizutani, Reo Yasuma, Nagaraj Kerur, Laura Tudisco, Shengjian Li, Tetsuhiro Yasuma
-
- 09:40** DARK REARING (DR) AS A MEANS OF MIMICKING PHYSIOLOGICAL HYPOXIA: A NON-INVASIVE INTERVENTION FOR RETINOPATHY OF PREMATURITY (ROP)
TAILOI CHAN-LING, Rita Maccarone, Mark Koina, Jennifer Lau, Peter Kozulin, Riccardo Natoli, Jan Provis, Silvia Bisti, Robert Linsenmeier, Samuel Adamson
-

13:00 – 15:00 RC05 – Photoreceptor Cell Biology I

Bayview A

Moderator: Orson Moritz

- 13:00** AXONEME ORGANIZATION AND IFT IN ZEBRAFISH AND MAMMALIAN PHOTORECEPTORS
JOSEPH BESHARSE, Tylor Lewis
-

13:20 **RAPID PHOTORECEPTOR DEGENERATION OCCURS IN ZEBRAFISH *ARL13B* MUTANTS FOLLOWING SUPPRESSION OF PLANAR CELL POLARITY SIGNALING**
BRIAN PERKINS, Ping Song

13:40 **OUTER SEGMENT PROTEIN DELIVERY RELIES ON A VARIETY OF TARGETING MOTIFS**
VADIM ARSHAIVSKY, Jillian Pearing, Raquel Salinas, Eric Lieu, Sheila Baker

14:00 **STRUCTURES AND PATHWAYS FOR MEMBRANE TARGETING IN RODS**
THEODORE WENSEL, Feng He, Melina Agosto, Zhixian Zhang, Michael Schmid

14:20 **RHODOPSIN TRAFFICKING AND MORPHOGENESIS OF OUTER SEGMENT DISK MEMBRANES**
DAVID WILLIAMS, Stefanie Volland, Vanda Lopes, Julian Esteve, Steven Fisher

14:40 **VISUALIZATION OF PROTEIN TRAFFICKING AND OUTER SEGMENT MORPHOGENESIS IN INTACT *XENOPUS LAEVIS* PHOTORECEPTOR CELLS**
YOSHIKAZU IMANISHI

16:30 – 18:30 RC06 – Role of Mononuclear Phagocytes in Age-Related Macular Degeneration

Moderator: Florian Sennlaub

Bayview B

16:35 **PHENOTYPE AND FUNCTION OF PERIPHERAL BLOOD MONOCYTE FROM AMD PATIENTS**
ITAY CHOWERS, Shira Hagbi-Levi, Michelle Grunin

17:00 **MONONUCLEAR PHAGOCYTE SURVIVAL IN THE SUBRETINAL SPACE**
FLORIAN SENNLAUB, Olivier Levy, Bertrand Calippe, Sophie Lavalette, Elisa Dominguez, Shulong Justin Hu, Christoph Combadière, Alexis-Pierre Bemelmans, José-Alain Sahel, Xavier Guillonneau

17:25 **NOVEL MARKERS AND THERAPY TARGETS FOR REACTIVE RETINAL MICROGLIA**
THOMAS LANGMANN

17:50 **MECHANISMS OF PHOTORECEPTOR TOXICITY OF MONONUCLEAR PHAGOCYTES**
XAVIER GUILLONNEAU, Shulong Hu, Bertrand Calippe, Sophie Lavalette, Florian Sennlaub

18:15 **REGULATING MACROPHAGE PHENOTYPE AND FUNCTION BY RETINAL PIGMENT EPITHELIAL CELLS**
MEI CHEN, Jiawu Zhao, Heping Xu

Wednesday, 23 July 2014

Lens

08:00 – 10:00 **LE07 – Lens Development**

Seacliff A

Moderators: Robb De Jongh, Michael Robinson

- 08:00 **THE STORY OF LENS: REGENERATION AND EVOLUTION**
PANAGIOTIS TSONIS
- 08:20 **LENS DEVELOPMENT IN ZEBRAFISH**
JEFFREY GROSS
- 08:40 **SPECIFICATION OF NEURAL RETINA IDENTITY BY LENS DERIVED BMP SIGNALING**
TANUSHREE PANDIT, Cedric Patthey, Lena Gunhaga, Vijay Kumar
- 09:00 **A NETWORK OF GROWTH FACTOR SIGNALING DETERMINES LENS POLARITY AND REGULATES ITS PRECISE THREE-DIMENSIONAL CELLULAR ARCHITECTURE**
JOHN MCAVOY, Lucy Dawes, Yuki Sugiyama, Frank Lovicu
- 09:20 **DNA METHYLTRANSFERASES IN MOUSE LENS DEVELOPMENT**
MICHAEL ROBINSON, Thanh Hoang, Devin Bruney, Savana Rosalez, Blake Rasor, Blake Chaffee, Evan Horowitz
- 09:40 **FUNCTION OF RNA BINDING PROTEINS IN LENS DEVELOPMENT**
SALIL LACHKE, Archana Siddam, Carole Gautier-Courteille, Atul Kakarana, Vincent Legagneux, Christine Dang, Linette Perez-Campos, Agnès Méreau, David Scheiblin, Justine Viet, David Beebe, Jeff Gross, Luc Paillard

Glaucoma

08:00 – 10:00 **GL07 – Blood Flow in Glaucoma**

Grand Ballroom

Moderators: Jeff Kiel, Herbert Reitsamer

- 08:00 **REGULATION OF BLOOD FLOW DEPENDENT ON BLOOD PRESSURE AND INTRAOCULAR PRESSURE**
LEOPOLD SCHMETTERER
- 08:20 **FUNCTIONAL OCT FOR GLAUCOMA**
DAVID HUANG
- 08:40 **VASCULAR AND CIRCULATORY CHANGES IN GLAUCOMA**
CHANDRAKUMAR BALARATNASINGAM, Dao-Yi D. Yu
- 09:00 **ENDOTHELIN-1 AND RETINAL VASOMOTOR REGULATION**
TRAVIS HEIN, Luke Potts, Robert Rosa, Jr., Lih Kuo
- 09:20 **OPTIC NERVE HEAD BLOOD FLOW RESPONSE TO INCREASED IOP AND DECREASED BP CHALLENGE IN EXPERIMENTAL GLAUCOMA**
LIN WANG, Grant Cull, Simon Thompson
- 09:40 **CONSIDERATIONS OF VENOUS PRESSURE IN THE PATHOPHYSIOLOGY OF INTRAOCULAR PRESSURE AND OCULAR BLOOD FLOW**
HERBERT REITSAMER, Clemens Strohmaier, Jeffrey Kiel

Cornea and Ocular Surface

08:00 – 10:00 **CO07 – Corneal Angiogenesis from Translational Aspects**

Seacliff D

Moderators: Claus Cursiefen, Dimitri Azar

- 08:00 **MMPS AND THEIR INHIBITION IN CORNEAL NEOVASCULARIZATION**
DIMITRI AZAR
- 08:20 **ANTILYMPHANGIOGENESIS IN CORNEAL TRANSPLANTATION AND DRY EYE DISEASE**
CLAUS CURSIEFEN
- 08:40 **MECHANISMS OF BLOOD VESSEL REGRESSION**
PAT D'AMORE
- 09:00 **TUMORS OF THE OCULAR SURFACE AND ANGIOGENESIS**
BERTIL DAMATO
- 09:20 **EFFECTS OF VEGF DEPLETION ON CORNEAL NERVES**
MARK ROSENBLATT, Victor Guaiquil, Zan Pan, Natalia Karagianni, Shima Fukuoka, Gemstonn Alegre
- 09:40 **INTEGRIN ALPHA 9 BLOCKADE INHIBITS LYMPHATIC VALVE FORMATION AFTER CORNEAL TRANSPLANTATION**
LU CHEN, Tan Truong, Gyeong Jin Kang, Eric Huang

Ocular Immunology

08:00 – 10:00 **IM04 – Ocular Infection and Host Defense**

Golden Gate

Moderator: Justine Smith

- 08:08 **C-TYPE LECTIN RECEPTORS: DYSFUNCTION OF HOST DEFENSE IN THE EYE?**
HOLLY ROSENZWEIG, Brianna Brown, Emily Vance, Phyllis Silver, Rachel Caspi, Ellen Lee
- 08:36 **GENERATION OF A HIGHLY EFFICACIOUS VACCINE AGAINST OCULAR HSV-1 INFECTION**
DAN CARR, Derek Royer, William Halford
- 09:04 **INTRACELLULAR *P. AERUGINOSA* VERSUS CORNEAL EPITHELIAL CELL: WHO WINS AND WHY?**
SUZANNE FLEISZIG, David Evans
- 09:32 **MECHANISMS OF *TOXOPLASMA GONDII* INFECTION OF HUMAN RETINA?**
IRA BLÄDER

Physiology and Pharmacology

08:00 – 10:00 **PH05 – Progress in Human Gene Therapy**

Bayview A

Moderator: Elizabeth Rakoczy

- 08:05 **RESPONSE OF THE VISUAL PATHWAYS IN THE HUMAN BRAIN TO GENE THERAPY**
JEAN BENNETT, Manzar Ashtari, Kathleen Marshall, Daniel Chung, Alberto Auricchio, Francesca Simonelli, Bart Leroy, Junwei Sun, Kenneth Schindler, Albert Maguire

08:28	GENE THERAPY FOR RETINOSCHISIS/PROGRESS IN HUMAN GENE THERAPY PAUL SIEVING
08:51	ONE YEAR FOLLOW-UP OF A PHASE 1 GENE THERAPY TRIAL WITH SUBRETINAL RAAV.SFLT-1 FOR THE LONG-TERM TREATMENT OF WET AGE-RELATED MACULAR DEGENERATION ELIZABETH RAKOCZY, Choo-May Lai, Aaron Magno, Cora Pierce, Thomas Chalberg, Stephen Schwartz, Mark Blumenkranz, Martin French, Ian Constable
09:14	EXPLORATION OF AAV-MEDIATED GENE THERAPIES FOR INHERITED OCULAR DISORDERS GWYNETH JANE FARRAR, Sophia Millington-Ward, Naomi Chadderton, Arpad Palfi, Mary O'Reilly, Fiona Mansergh, Matthew Carrigan, Paul Kenna
09:37	GENE THERAPY FOR USHER 1B: STUDY ON MYO7A ISOFORMS AND DOMINANT MUTATIONS VANDA LOPES, Agrani Rump, Mei Jiang, David Williams

Ocular Imaging

08:00 – 10:00 OI05 – Fusion of Imaging and Bioengineering For Eye Disease Marina
Moderators: Dan Stamer, C. Ross Ethier

08:00	TRABECULAR MESHWORK STRESS-STRAIN RESPONSE IN LIVING VERSUS DONOR EYES: A PILOT STUDY LARRY KAGEMANN, Bo Wang, Gadi Wollstein, Hiroshi Ishikawa, Ian Sigal, Joel J. Schuman
08:24	TOWARDS IMPROVED GLAUCOMA MANAGEMENT: MAPPING OPTIC NERVE HEAD BIOMECHANICS USING SPECTRAL-DOMAIN OCT, MICRO-OCT, AND ADAPTIVE OPTICS MICHAEL GIRARD, Khai Sing Chin, Meghna Beotra, Linbo Liu, Nicholas Strouthidis
08:48	VASCULAR MAPPING WITH AN AOSLO STEPHEN BURNS, Gang Huang, Alberto deCastro, Lucie Sawides, Ting Luo, Thomas Gast
09:12	CHOROIDAL OCT WOLFGANG DREXLER, Marieh Esmaeelpour
09:36	NEW FINDINGS ON SCLERAL COLLAGEN ARCHITECTURE USING MICRON-SCALE FIBER ORIENTATION ANALYSIS IAN SIGAL, Jonathan Grimm, Ning-Jiun Jan, Huong Tran, Celeste Gomez, Gadi Wollstein, Hiroshi Ishikawa, Joel Schuman, Larry Kagemann

Retinal Neuroscience and Development

08:00 – 10:00 RN07 – Cell-to-Cell Signaling and Retinal Development Seacliff B
Moderator: Sabine Fuhrmann

08:05	VASCULAR ENDOTHELIAL CELLS ARE REQUIRED FOR NORMAL RETINAL NEUROGENESIS IN THE ZEBRAFISH DEBORAH STENKAMP, Susov Dhakal, Craig Stevens, Omri Weiss, Adi Inbal
08:30	INTERCELLULAR SIGNALING AND MAINTENANCE OF MORPHOGEN RESPONSIVENESS IN THE DEVELOPING RETINA VALERIE WALLACE

08:55	RELATIONSHIPS BETWEEN ORGANELLE DYNAMICS AND POLARIZED SIGNALING DURING RETINAL DEVELOPMENT BRIAN LINK
09:20	ROLES OF WNT SIGNALING DURING EYE DEVELOPMENT SABINE FUHRMANN, Mary Colasanto, Kayla Dyorich, Milan Jamrich, Charles Murtaugh, Eliza Bankhead
09:40	REPROGRAMMING OF THE CHICK RETINAL PIGMENTED EPITHELIUM AFTER RETINAL INJURY KATIA DEL RIO-TSONIS, Agustin Luz-Madrigal, Erika Grajales-Esquivel, Alexander McCorkle, Karla Barbosa-Sabanero, Panagiotis Tsonis

Retinal Cell Biology

08:00 – 10:00	RC07 – Unfolded Protein Response in Oxidative Stress in Retinal Degenerations Moderators: Steven Fliesler, Sarah Zhang	Bayview B
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08:05	ROLE OF THE UNFOLDED PROTEIN RESPONSE IN REGULATION OF RPE CELL SURVIVAL SARAH ZHANG
08:30	THE ROLE OF ADVANCED GLYCATION/LIPOXIDATION IN THE PATHOGENESIS OF DIABETIC RETINOPATHY ALAN STITT
08:55	ENDOPLASMIC RETICULUM-ASSOCIATED DEGRADATION (ERAD) OF MISFOLDED RHODOPSIN DISRUPTS PHOTORECEPTOR PROTEIN HOMEOSTASIS IN RETINAL DEGENERATION JONATHAN LIN, Wei-Chieh Chiang, Heike Kroeger, Carissa Messah, Doug Yasumura, Michael Matthes, Sanae Sakami, Judith Copping, Krzysztof Palczewski, Matthew LaVail
09:20	MODULATION OF THE UNFOLDED PROTEIN RESPONSE IN ADRP RETINA: POTENTIAL THERAPEUTIC IMPLICATIONS MARINA GORBATYUK
09:45	PROTECTION OF HUMAN RPE CELLS FROM ER STRESS BY HUMANIN: RELATIONSHIP TO ANTIOXIDANT ENZYMES AND MITOCHONDRIAL GSH DOUGLAS MATSUNAGA, Keijiro Ishikawa, Sreekumar Parameswaran, Ram Kannan, David Hinton

Joint Session

08:00 – 10:00	JT01 – RPE and Photoreceptor Biology Moderators: Hui Sun, Muna Naash	Seacliff C
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08:00	SMALL PEPTIDES FROM PEDF THAT BIND PEDF-R AND PROTECT THE RETINA S. PATRICIA BECERRA, Jason Kenealey, Preeti Subramanian, Antonella Comitato, Valeria Marigo
08:12	IDENTIFICATION AND CHARACTERIZATION OF THE TRANSMEMBRANE RECEPTORS FOR A SECRETED FACTOR WITH BROAD THERAPEUTIC VALUES HUI SUN, Guo Cheng, Ming Zhong, Riki Kawaguchi, Miki Kassai, Jun Deng, Mariam Ter-Stepanian
08:32	LOSS OF RETINITIS PIGMENTOSA 2 (RP2) PROTEIN MODULATES MICROTUBULE DYNAMICS AND CONE PHOTORECEPTOR OUTER SEGMENT EXTENSION HEMANT KHANNA, Linjing Li, Kollu Rao, Yun Zheng-Le, Toby Hurd, Concepcion Delgado

08:52	VARYING PATHOBIOLOGY FOR PRPH-2-ASSOCIATED DISEASE MUNA NAASH
09:12	THE CELL POLARITY PROTEINS CRB2 AND CRB3 ARE EXPRESSED IN THE RPE ANTONIO ESCUDERO, David Jimeno, Angela Jimeno, Almudena Velasco, Concepcion Lillo
09:24	OCULAR ALBINISM: INSIGHTS ON THE EXPRESSION OF RETINAL GENES REGULATED BY OA1 ALEJANDRA YOUNG,, Sonia Guha, Novrouz Akhmedov, Deborah Farber
09:36	αB-CRYSTALLIN REGULATES EXOSOMES SECRETION IN RETINAL PIGMENT EPITHELIAL CELLS SURAJ BHAT, Rajendra Gangalum, Sirius Kohan
09:48	DJ-1 EXPRESSION LEVELS PERTURB MITOCHONDRIA STRUCTURE IN THE RETINAL PIGMENT EPITHELIUM (RPE) VERA BONILHA, Mary Rayborn, Karen Shadrach

Thursday, 24 July 2014

Lens

08:00 – 10:00 LE08 – Signaling Pathways Determining Lens Cell Fate and Differentiation Seacliff A
Moderators: Linda Musil, Frank Lovicu

08:00	SYNERGISTIC INTERACTION BETWEEN THE FGF AND BMP SIGNALING PATHWAYS IN LENS CELLS LINDA MUSIL
08:20	PROX1 TRANSCRIPTIONAL UPREGULATION OF FIBROBLAST GROWTH FACTOR RECEPTOR EXPRESSION IS REQUIRED FOR LENS FIBER DIFFERENTIATION DYLAN AUDETTE, Melinda Duncan
08:40	FGFR-ERK SIGNALING IN LENS, CORNEAL EPITHELIUM AND LACRIMAL GLAND DEVELOPMENT LIXING RENEKER, Dinesh Upadhya, Jinglin Zhang
09:00	PI3K SIGNALING IN LENS DEVELOPMENT XIN ZHANG
09:20	PI3K, PTEN AND AKT IN LENS GROWTH AND HOMEOSTASIS THOMAS WHITE, Leping Li, Richard Lin, Caterina Sellitto
09:40	NEGATIVE REGULATORS OF GROWTH FACTOR SIGNALING IN THE LENS FRANK LOVICU, Guannan Zhao, Fatima Wazin, Ana Cham, Tammy So, Magdalena Wojciechowski, John McAvoy

13:00 – 15:00 LE09 – PCO: Can We Prevent Secondary Cataract? Seacliff A
Moderators: Janice Walker, Judy West-Mays

13:00	THE ROLE OF ECM IN POSTERIOR CAPSULAR OPACIFICATION (PCO) MELINDA DUNCAN, Mallika Pathania, Saleena Mallik, Yan Wang
13:20	POTENTIAL SIGNALING PATHWAYS TO TARGET IN PCO JUDITH WEST-MAYS, Madhuja Gupta, Anna Korol

13:40	THE ROLE OF REPAIR CELLS IN WOUND HEALING AND FIBROSIS IN A LENS CATARACT SURGERY MODEL JANICE WALKER, Sue Menko, Brigid Bleaken
14:00	REGULATION OF LENS FORMATION AND MAINTENANCE BY PRIMARY CILIA YUKI SUGIYAMA, Elizabeth Shelley, Li Wen, Bradley Yoder, Michael Robinson, Zbynek Kozmik, Frank Lovicu, John McAvoy
14:20	UNDERSTANDING THE BASIS OF OPEN CAPSULAR BAG STRATEGIES IN THE PREVENTION OF PCO MICHAEL WORMSTONE, Julie Eldred, David Spalton
14:40	MMP INHIBITOR DELIVERY AND MODIFICATION AS A POTENTIAL MEANS OF MITIGATING PCO HEATHER SHEARDOWN, Judith West-Mays, Diana Morarescu, Bahram Amoozgar

16:30 – 18:30 **LE10 – Molecular Basis for Lens Architecture and Functional Quality**

Moderators: Xiaoua Gong, Matthew Reilly

Seacliff A

16:30	CYTOSKELETAL SYNERGY IN LENS FUNCTION VELIA FOWLER, Roberta Nowak
16:47	CONTRIBUTIONS OF INTERMEDIATE FILAMENT PROTEINS TO LENS BIOLOGY PAUL FITZGERALD, John Hess, Vijay Krishna Raghunathan, Doug Fudge, Cecilia Boutry
17:04	LENS FIBER CELL MATURATION INVOLVES UNIQUE CELLULAR PATTERNS AND SUB-CELLULAR STRUCTURES MARTIN COSTELLO, Kurt Gilliland, Ashik Mohamed, Sönke Johnsen, Kevin Schey
17:21	LENS FUNCTION RESULTS FROM THE PERFECT FUSION OF CELL SHAPE AND THEIR GROWTH ROY QUINLAN, Weiju Wu, Miguel Jarrin, Laura Young, Chris Saunter, John Girkin, Jim Hall, Daniel Clemens
17:38	THE DECORATION OF FIBER CELL MEMBRANE CYTOSKELETAL-LINKED DOMAINS AND THEIR ROLE IN LENS CYTOARCHITECTURE AND TENSILE PROPERTIES RUPALATHA MADDALA, Vasanth Rao
17:55	SYNERGISTIC EFFECTS OF INTERCELLULAR GAP JUNCTION COMMUNICATION AND MEMBRANE-CYTOSKELETON NETWORK IN LENS TRANSPARENCY AND STIFFNESS XIAOHUA GONG, Chun-Hong Xia, Mei Li, Deepika Arora, Eddie Wang, Wiktor Stopka, Catherine Cheng, Jing Zeng
18:12	DIRECT AND INDIRECT MEASUREMENTS OF LENS MECHANICAL PROPERTIES MATTHEW REILLY, Saurav Kumar, Matthew Leroux, Andrew Shiels, Brenda Marchand

Glaucoma

08:00 – 10:00 **GL08 – Aqueous Humor Dynamics**

Moderators: Arthur Sit, Makoto Aihara

Grand Ballroom

08:00	MEASUREMENT OF AQUEOUS HUMOR FLOW: PRINCIPLES, PITFALLS AND PATTERNS ARTHUR SIT, Jay McLaren
08:20	EPISCLERAL VENOUS PRESSURE – MEASUREMENT AND REGULATION JEFFREY KIEL

08:40	UVEOLYMPHATIC OUTFLOW PATHWAY YENI YUCEL, Neeru Gupta
09:00	THE EFFECTS OF VITRECTOMY AND CATARACT SURGERY ON OXYGEN LEVELS AND DISTRIBUTION IN RHESUS MONKEY EYES PAUL KAUFMAN, Ying-Bo Shui, David Beebe, Baohe Tian, Gregg Heatley, T. Michael Nork, Carla Siegfried
09:20	AQUEOUS HUMOR DYNAMIC STRUCTURAL MARKERS OF ONE-YEAR ANGLE SURGERY OUTCOMES SAYOKO MOROI, Samir Shah, Surbhi Bansal, Jesse Gilbert, David Reed, Alan Argento, Nilay Chakraborty
09:40	DO WE NEED A NEW GOLDMANN EQUATION? CAROL TORIS

13:00 – 15:00	GL09 – IOP-Lowering Agents Moderators: Ganesh Prasanna, Xuyang Liu	Grand Ballroom
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13:00	CAVEOLAE AND CONVENTIONAL OUTFLOW: PROTEOMIC PROFILING OF OUTFLOW TISSUE CAVEOLAE AND FUNCTIONAL STUDIES IN CAVEOLAE-DEFICIENT MICE MICHAEL ELLIOTT, Stefanie Hauck, Nicole Ashpole, Xiaowu Gu, Mark McClellan, Mikhail Dozmorov, Jonathan Wren, Masaki Tanito, Ernst Tamm, Daniel Stamer
13:20	EFFECT OF INFLAMMATORY CYTOKINES ON AQUEOUS OUTFLOW TOSHIHIRO INOUE
13:40	HYDROGEL SUSTAINED-RELEASE OF C3 EXOENZYME LOWERED IOP IN OCULAR HYPERTENSIVE RATS XUYANG LIU, Zhi Wang, Lu Liu, Jie Yang, Iok-Hou Pang
14:00	AUTOTAXIN IS A DRUGGABLE MOLECULAR TARGET TO LOWER IOP AND A POTENTIAL BIOMARKER FOR GLAUCOMA VASANTH RAO
14:20	HIGH THROUGHPUT SCREEN FOR THE IDENTIFICATION OF AGENTS THAT RELAX SCHLEMM'S CANAL ENDOTHELIAL CELLS AND INCREASE OUTFLOW FACILITY DANIEL STAMER, Enhua Zhou, Chan Park, James Butler, Kristin Perkumas, Mark Johnson, Jeffrey Fredberg
14:40	SOLUBLE GUANYLATE CYCLASE: AN EMERGING THERAPEUTIC TARGET IN PRIMARY OPEN ANGLE GLAUCOMA EMMANUEL BUYS, Peter Brouckaert, Janey Wiggs, Meredith Gregory, Louis Pasquale, Kenneth Bloch, Bruce Ksander

16:30 – 18:30	GL10 – Hot Topics in Glaucoma Moderators: Colm O'Brien Mater, Abe Clark	Grand Ballroom
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16:30	GENETIC AND PHARMACOLOGICAL REDUCTION OF ER STRESS IN THE TRABECULAR MESHWORK RESCUES GLAUCOMA IN MOUSE MODELS OF GLAUCOMA GULAB ZODE
16:54	EPIGENETIC REGULATION OF GLAUCOMA-ASSOCIATED FACTORS IN THE TRABECULAR MESHWORK WEIMING MAO, Jaclyn Bermudez, Hannah Webber, Abbot Clark
17:18	GLAUCOMA: AN INSIGHT INTO EPIGENETIC REPROGRAMMING DEBORAH WALLACE, Fiona McDonnell, Abbot Clark, Colm O'Brien

- 17:42 **GENETIC ANALYSIS OF SUSCEPTIBILITY TO RETINAL GANGLION CELL LOSS USING INBRED MICE: THE STORY OF SPINK2**
ROBERT NICKELLS, Cassandra Schlamp
- 18:06 **EXCITOTOXIC RGC DEATH DOES NOT REQUIRE JUN OR TNF**
KIMBERLY FERNANDES, Berkeley Fahrenthold, Rebecca Rausch, Richard Libby

Cornea and Ocular Surface

08:00 – 10:00 CO08 – Novel Corneal Imaging Technologies from Basic Science to Clinical Application
Moderators: James Jester, Rudolf Guthoff

Seacliff D

- 08:00 **MODALITIES OF MULTIPHOTON IMAGING IN VIVO**
ALEXANDER HEISTERKAMP, Tobias Ehmke, Andreas Knebl, Franck Gounou, Tim Nitzsche, Ronny Grosse, Maria Reichard, Heike Weiss, Simone Baltrusch, Oliver Stachs
- 08:20 **3-DIMENSIONAL ASSESSMENT OF THE CORNEAL RESPONSE TO INJURY AND DISEASE *IN VIVO* USING A MODIFIED HRT-RCM CONFOCAL MICROSCOPE**
WALTER PETROLL, Danielle Robertson, H. Dwight Cavanagh
- 08:40 **LARGE SCALE IN VIVO IMAGING OF THE CORNEAL SUB-BASAL NERVE PLEXUS**
BERND KÖHLER, Stephan Allgeier, Susanne Maier, Klaus-Martin Reichert, Sabine Peschel, Rudolf Guthoff, Oliver Stachs, Georg Bretthauer
- 09:00 **IMMUNOFLUORESCENT TOMOGRAPHY OF OCULAR SURFACE ADULT STEM CELLS**
GERAINT PARFITT, James Jester
- 09:20 **THREE-DIMENSIONAL MATRIX ULTRASTRUCTURE OF THE DEVELOPING CORNEA REVEALED BY SERIAL BLOCK FACE SCANNING ELECTRON MICROSCOPY**
ANDREW A. QUANTOCK, Carlo Knupp, Tobias Starborg, Karl Kadler, Robert Young
- 09:40 **ADVANCED BRILLOUIN SPECTROSCOPIC TECHNIQUES**
STEPHAN REISS, Rudolf Guthoff, Heinrich Stoltz, Oliver Stachs

13:00 – 15:00 CO09 – Corneal Tissue Engineering and Gene Therapy
Moderators: Kohji Nishida, Choun-Ki Joo

Seacliff D

- 13:00 **STRATEGIES OF HUMAN CORNEA TISSUE REGENERATION**
CHOUN-KI JOO
- 13:15 **BIOSYNTHETIC CORNEAS AS REGENERATION TEMPLATES: AN UPDATE**
MAY GRIFFITH, Jaywant Phopase, Oleksiy Buznyk
- 13:30 **BIO-PROSTHESIS: A BOTTOM-UP APPROACH TO CORNEAL TISSUE ENGINEERING**
CHE CONNON, Ricardo Gouveia, Valeria Castelletto, Ian Hamley
- 13:45 **SILK FOR CORNEAL TISSUE ENGINEERING**
GILSON KHANG, Eun Young Kim, Nirmalya Tripathy, Jeong Eun Song, Choun-Ki Joo, Dongwon Lee
- 14:00 **BIOMATERIALS FOR REPAIR AND REGENERATION OF EYE TISSUES**
JENNIFER ELISSEFF, Qiongyu Guo, Shoumyo Majumdar, Jeremy Chae

- 14:15** **OCULAR SURFACE RECONSTRUCTION WITH ORAL MUCOSAL EPITHELIAL CELL SHEETS**
YOSHINOBU OIE, Kohji Nishida
-
- 14:30** **MATURATION OF HUMAN CORNEAL ENDOTHELIAL CELLS CULTURED ON SELF-ASSEMBLED FIBROBLAST MATRIX**
JAMES ZIESKE, Xiaoqing Guo, Audrey Hutcheon
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- 14:40** **MICRO RNAS EXPRESSED IN NORMAL AND DIABETIC HUMAN CORNEAS**
MEHRNOOSH SAGHIZADEH, Michael Winkler, Christian Dib, Jordan Brown, Jie Tang, Lindsay Spurka, Vincent Funari, Alexander Ljubimov
-

16:30 – 18:30 **CO10 – Somatic and Pluripotent Stem Cell Technology: Towards the Corneal Regeneration**

Seacliff D

Moderators: Alexander Ljubimov, Sophie Deng

- 16:30** **GENE AND CELL THERAPY APPROACHES FOR CORNEAL EPITHELIAL DISORDERS**
ALEXANDER LJUBIMOV, Andrei Kramerov, Michael Winkler, Tanya Spektor, Mehrnoosh Saghizadeh
-
- 16:50** **NOVEL APPROACHES TO BIOENGINEERING LIMBAL STEM / PROGENITOR CELLS**
SOPHIE DENG
-
- 17:10** **INDUCED PLURIPOTENT STEM CELL TECHNOLOGY FOR THE TREATMENT OF CORNEAL ENDOTHELIAL DISEASE**
SHIGETO SHIMMURA
-
- 17:30** **CELL BASED APPROACH FOR THE TREATMENT OF CORNEAL ENDOTHELIAL DYSFUNCTION**
NORIKO KOIZUMI
-
- 17:50** **CORNEAL EPITHELIAL STEM CELLS AND NICHE MICROENVIRONMENTS**
URSULA SCHLÖTZER-SCHREHARDT, Friedrich Kruse
-
- 18:10** **STEM CELL AND CORNEAL REGENERATIVE MEDICINE**
KOHJI NISHIDA
-

Ocular Immunology

08:00 – 10:00 **IM05 – Pathogenic Antibodies in Ocular Disease**

Seacliff C

Moderator: John Curnow

- 08:00** **INTRODUCTION TO PATHOGENIC ANTIBODIES IN OCULAR DISEASE**
JOHN CURNOW
-
- 08:05** **AUTOREACTIVE T AND B CELLS IN DRY EYE DISEASE**
MICHAEL STERN, Christopher Schaumburg, Jianping Gao, Larry Wheeler, Margarita Calonge, Jerry Niederkorn, Stephen S. Pflugfelder
-
- 08:30** **AUTOANTIBODIES TO INSULIN-LIKE GROWTH FACTOR-1 RECEPTOR IN THYROID-ASSOCIATED OPHTHALMOPATHY**
JOHN CURNOW, Matthew Edmunds
-
- 08:55** **AUTOANTIBODIES IN GLAUCOMA**
FRANZ GRUS
-

09:20

ROLE OF ANTI-RETINAL AUTOANTIBODIES IN PARANEOPLASTIC RETINOPATHY
GRAZYNA ADAMUS

Physiology and Pharmacology

08:00 – 10:00 PH06 – Novel Engineered Therapies for Glaucoma

Marina

Moderators: Dan Stamer, C. Ross Ethier

08:05

GLAUCOMA THERAPY BY CONTACT LENSES WITH VITAMIN E BARRIERS
ANUJ CHAUHAN

08:28

EYEWATCH: A NOVEL ADJUSTABLE GLAUCOMA DRAINAGE DEVICE
SYLVAIN ROY, Adan Villamarin, Alexandre Moulin, André Mermoud, Nikos Stergiopoulos

08:51

CAN REPLACEMENT OF DAMAGED OR LOST TRABECULAR MESHWORK CELLS RESTORE IOP CONTROL IN GLAUCOMA?
MARKUS KUEHN

09:14

TRABECULAR MESHWORK STEM CELLS AND CELL-BASED THERAPY FOR GLAUCOMA
YIQIN DU

09:37

INTRAVITREAL NEURAL STEM CELLS FROM HUMAN ADULT TEMPORAL LOBES ARE NEUROPROTECTIVE IN A RAT MODEL OF OPTIC NERVE TRANSACTION
SANG JIN KIM, Ji-Hyun Yun

RPE Biology and Pathology

08:00 – 10:00 RP07 – NLRP3 Inflammasome

Bayview A

Moderator: Matthew Campbell

08:05

IL-18 PROCESSING AND AGE-RELATED MACULAR DEGENERATION: A TALE OF TWO STATES
SARAH DOYLE, Ema Ozaki, Marian Humphries, Pete Humphries, Pete Adamson, Matthew Campbell

08:28

VEGF-A AND THE NLRP3 INFLAMMASOME IN AGE-RELATED MACULAR DEGENERATION
ALEXANDER MARNEROS

08:51

NLRP3 INFLAMMASOME, IL-1 β /IL-18, AND IL-17 IN AGE-RELATED MACULAR DEGENERATION
CHI-CHAO CHAN, Yujuan Wang

09:14

THE INFLAMMASOME AS THE INTEGRATOR OF MULTIPLE AMD PATHOGENIC STIMULI
BRADLEY GELFAND, Jayakrishna Ambati

09:37

NLRP3 INFLAMMASOME SIGNALING IN HUMAN RPE CELLS
ANU KAUPPINEN

13:00 – 15:00 RP08 – AMD Genetics, Pathogenesis, and Stem Cells

Bayview A

Moderator: Kang Zhang

13:05

INFLAMMASOME IN AMD
JAYAKRISHNA AMBATI

13:33	OXIDATIVE STRESS IN STEM CELL-DERIVED RPE CELLS: IMPLICATIONS FOR AMD DEEPAK LAMBA
14:01	GENETICS AND STEM CELL BASED THERAPIES FOR MACULAR DEGENERATION KANG ZHANG, Huimin Cai, Alice Song
14:29	INTERVENTION OF RETINAL DEGENERATION IN RCS RATS WITH SUBRETINAL TRANSPLANTATION OF ADIPOSE-DERIVED STEM CELLS GUO-TONG XU, Zongyi Li, Lixia Lu, Juan Wang, Jieping Zhang, Haibin Tian, Furong Gao, Fang Wang

16:30 – 18:30 **RP09 – Generating RPE from Stem Cells**

Bayview A

Moderators: Peter Westenskow, Michael Boulton

16:35	A SYSTEMIC THERAPY FOR DRY AMD USING PROGRAMMED HEMATOPOIETIC STEM CELLS MICHAEL BOULTON, Xiaoping Qi, Yuanqing Yan, Louise Pay, Lynn Shaw, Alfred Lewin, Maria Grant
16:55	DEFINED PRODUCTION OF HUMAN EMBRYONIC STEM CELL-DERIVED RETINAL PIGMENTED EPITHELIAL CELLS AS A CELL-BASED TREATMENT FOR AGE-RELATED MACULAR DEGENERATION SHERRY HIKITA, Britney Pennington, Dennis Clegg, Lincoln Johnson
17:15	RETINAL SHEET TRANSPLANTS REPLACE PHOTORECEPTORS AND RESTORE VISION MAGDALENE SEILER, Robert Aramant, Biju Thomas, Pamela Yang, Norman Radtke, Hans Keirstead
17:30	SELECTIVE IMMUNOGENICITY OF IPS-DERIVED CELLS PETER WESTENSKOW, Martin Friedlander
17:50	MAKING RPE FROM HIPSC – CONSIDERATIONS FOR DISEASE MODELING RUCHIRA SINGH, David Kuai, Jackelyn Meyer, David Gamm
18:10	PATIENT-SPECIFIC IPSC-BASED CONFIRMATION OF A PATHOGENIC CRYPTIC SPLICE SITE MUTATION IN RPE65 ENABLES PATIENT ENROLLMENT IN GENE AUGMENTATION TRIAL BUDD TUCKER, Cathryn Cranston, Kristin Anfinson, Dalyz Ochoa, Robert Mullins, Edwin Stone

Retinal Neuroscience and Development

08:00 – 10:00 **RN08 – Retinal Degeneration Genetics and Mechanisms**

Seacliff B

Moderator: Takeshi Iwata

08:00	UNDERSTANDING THE MOLECULAR BASIS OF RD BY EXOME ANALYSIS RADHA AYYAGARI, Bruno Maranhao, Pooja Biswas, Jacque Duncan, Igor Kozak, S. Amer Riazuddin
08:24	HIGHLY PENETRANT ALLELES IN AGE-RELATED MACULAR DEGENERATION ANNEKE DEN HOLLANDER
08:48	COMPREHENSIVE GENETIC ANALYSIS IN INHERITED RETINAL DISEASES APPLYING NEXT-GENERATION SEQUENCING CHRISTINA ZEITZ, Saïd El Shamieh, Marion Neuillé, Angélique Terray, Elise Orhan, Thierry Léveillard, Saddek Mohand-Saïd, Olivier Goureau, José-Alain Sahel, Isabelle Audo
09:12	CHARACTERIZATION OF NOVEL GENES RESPONSIBLE FOR HEREDITARY RETINAL DISEASES IN JAPANESE POPULATION TAKESHI IWATA

09:36 **MUTATIONS IN COL4A1 CAUSE RETINAL VASCULAR LESIONS**
MARCEL ALAVI, Douglas Gould

13:00 – 15:00 RN09 – Epigenetics in Development and Diseases
Moderator: Seth Blackshaw

Seacliff B

13:05 **POLYCOMB REGULATION OF RETINAL PROGENITOR PROLIFERATION AND DIFFERENTIATION**
MONICA VETTER

13:35 **3-D CHROMATIN ORGANIZATION OF MURINE PHOTORECEPTORS**
SHIMING CHEN, Philip Ruzycski

14:05 **ON THE VERGE OF NEURONAL REPLACEMENT: CELLULAR PLIANCY AND RETHINKING
AGE-OLD DOGMA**
MIKE DYER

14:35 **HYBRID MICE REVEAL PARENT-OF-ORIGIN AND *CIS*- AND *TRANS*-REGULATORY EFFECTS
IN THE RETINA**
SUSAN SHEN, Ernest Turro, Joseph Corbo

**16:30 – 18:30 RN10 – Road to Cure II: Gene Replacement, Optogenetics,
and Other Therapies for Retinal Diseases**
Moderator: Zhuo-Hua Pan

Seacliff B

16:35 **RESTORATION OF THE MAJORITY OF THE VISUAL SPECTRUM IN RCS RATS USING AAV-MEDIATED
MODIFIED *VOLVOX* CHANNELRHODOPSIN-1 GENE TRANSFER**
HIROSHI TOMITA, Eriko Sugano, Namie Murayama, Kitako Tabata, Maki Takahashi, Takehiko Saito,
Fumiaki Nishiyama, Makoto Tamai

17:00 **RESTORING VISUAL FUNCTION BY ECTOPIC EXPRESSION OF MELANOPSIN TO RETINAL BIPOLAR
CELLS IN RETINAL DEGENERATION**
BIN LIN

17:25 **RESTORING VISUAL FUNCTION TO BLIND MICE WITH CHEMICAL PHOTOSWITCHES THAT EXPLOIT
ELECTROPHYSIOLOGICAL REMODELING OF THE DEGENERATING RETINA**
RICHARD KRAMER, Ivan Tochitsky

17:50 **EVALUATION AND DEVELOPMENT OF MORE LIGHT-SENSITIVE CHR2 MUTANTS FOR
VISUAL RESTORATION**
ZHUO-HUA PAN, Tushar Ganjawala, Qi Lu, Elena Ivanova, Zhifei Zhang

18:15 **AN NRF2 DERIVED PEPTIDE DELIVERED BY AN AAV VECTOR PROTECTS THE EYE AGAINST
INFLAMMATION AND OXIDATIVE STRESS**
CRISTHIAN ILDEFONSO, Henrique Jaime, Qihong Li, Alfred Lewin

Retinal Cell Biology

08:00 – 10:00 RC08 – Photoreceptor Cell Biology II
Moderators: Joe Besharse, David Williams

Bayview B

08:05 **RHODOPSIN TRAFFICKING PROTEINS AND RETINAL DEGENERATIONS**
ALECIA GROSS, Evan Boitet, Nicholas Reish

- 08:28 **RHODOPSIN ACTIVELY RECRUITS THE SORTING MACHINERY THAT REGULATES ITS TRAFFICKING TO THE CILIA**
DUSANKA DERETIC, Jing Wang
-
- 08:51 **THE ROLE OF CA_v1.4 CHANNELS IN THE DEVELOPMENT OF SYNAPTIC RIBBONS**
SHEILA BAKER, Vasily Kerov, Xiaoni Liu, Joseph Laird, Brittany Williams, Amy Lee
-
- 09:14 **MULTIPLE FATES OF MUTANT P23H RHODOPSIN IN DEGENERATING PHOTORECEPTORS**
ORSON MORITZ, Beatrice Tam, Tami Boga
-
- 09:37 **RHODOPSIN-MEDIATED PHOSPHOLIPID FLIP-FLOP: IMPLICATIONS FOR A2E SYNTHESIS**
ANANT MENON, Michael Goren
-

13:00 – 15:00 RC09 – Cell Polarity and Signaling during Ocular Development Bayview B
Moderator: Brian Link

- 13:00 **REGULATION OF MITOTIC SPINDLE ORIENTATION AND ASYMMETRIC CELL DIVISIONS IN THE DEVELOPING MOUSE RETINA**
MICHEL CAYOUE
-
- 13:30 **LHX2 CHIP-SEQ ANALYSIS IDENTIFIES TARGET GENES CONTROLLING PROGENITORS MAINTENANCE AND LINEAGE COMMITMENT IN EARLY POSTNATAL MURINE RETINA**
CRISTINA ZIBETTI, Jianfei Hu, Woonchang Hwang, David O'Brien, Hao Zhang, Jiang Qian, Seth Blackshaw
-
- 14:00 **POLARITY REGULATORS AND CANCER IN THE MURINE RETINA**
ROD BREMNER, Mohammad Ahmad, Arthur Aubry
-
- 14:30 **DISTINCT DISTRIBUTIONS AND FUNCTIONS OF THREE CRUMBS (CRB) PROTEINS IN THE ZEBRAFISH RETINA**
XIANGYUN WEI
-

16:30 – 18:30 RC10 – Signaling Sphingolipids in Ocular Diseases Bayview B
Moderators: Nawajes Mandal, Sanjoy Bhattacharya

- 16:35 **SPHINGOLIPID-MEDIATED INFLAMMATION AT THE OCULAR SURFACE**
ALEXANDRA ROBCIUC, Matti Jauhiainen, Juha Holopainen
-
- 17:00 **SPHINGOSINE-1-PHOSPHATE SIGNALING IN RETINA PHOTORECEPTORS AND GLIAL CELLS**
NORA ROTSTEIN, Victoria Simon, Facundo Prado Spalm, Daniela Agnolazza, Nawajes Mandal, Luis Politi
-
- 17:25 **COMPARISON OF VITREOUS AND SERUM SPHINGOLIPIDS IN PATIENTS WITH PROLIFERATIVE DIABETIC RETINOPATHY TO PATIENTS WITHOUT RETINAL VASCULAR DISEASE**
LOUIS GLAZER, Todd Lydic, Julia Busik, Gavin Reid
-
- 17:45 **OVEREXPRESSION OF ELOVL4 PREVENTS DIABETES-INDUCED BLOOD-RETINAL BARRIER BREAKDOWN THROUGH AN INCREASE IN VERY LONG CHAIN CERAMIDES**
NERMIN KADY, Xuwen Liu, Todd Lydic, Sergey Seregin, Andrea Amalfitano, Sanford Boye, William Hauswirth, David Antonetti, Julia Busik, Vince Chiodo
-
- 18:05 **REVERSE GENETICS TO UNDERSTAND THE ROLE OF SPHINGOLIPID IN THE RETINA**
NAWAJES MANDAL, Madeline Budda, Hui Qi, Megan Stiles, Tuan-Phat Huynh, William Johnson
-

Joint Sessions

13:00 – 15:00 JT02 – Ion Channels in Eye Disease

Seacliff C

Moderators: Xavier Gasull, Olaf Strauss

- 13:00 ION CHANNELS IN CORNEAL SENSORY INNERVATION: ROLE IN OCULAR PATHOLOGIES
XAVIER GASULL, Gerard Callejo, Aida Castellanos, Carolina Luna, Susana Quirce, Illes Kovács, Maria Carmen Acosta, Carlos Belmonte, Juana Gallar, Jonathan Giblin
- 13:30 MECHANOSENSITIVE CHANNELS OF TRABECULAR MESHWORK
SANJOY BHATTACHARYA, Teresia Carreon, Carmen Piqueras, Aida Castellanos, Xavier Gasull
- 14:00 RETINAL CNG CHANNELOPATHIES: FROM MECHANISMS TO TREATMENTS
STYLIANOS MICHALAKIS
- 14:30 BESTROPHIN-1, AN INTRACELLULAR CL CHANNEL OF THE RETINAL PIGMENT EPITHELIUM
OLAF STRAUSS, Claudia Müller, Nestor Mas Gomez

16:30 – 18:30 JT03 – The New Concept of the Blood–Aqueous Barrier

Seacliff C

Moderator: Thomas Freddo

- 16:30 NOT ALL CLINICALLY OBSERVABLE FLARE IS PATHOLOGICAL
THOMAS FREDDO, Haiyan Gong
- 17:00 THE MAGNITUDE OF THE UVEOSCLERAL INFLOW PATHWAY IN THE HUMAN EYE: EVIDENCE FROM PATIENTS WITH CORNEAL ENDOTHELIAL DYSTROPHY
DAVID BEEBE, Andrew Huang, Ying-Bo Shui, Carla Siegfried, Benjamen Filas, Fang Bai
- 17:30 BETWEEN THE BLOOD AND AQUEOUS HUMOR: DEFINING A THIRD COMPARTMENT IN BLOOD–AQUEOUS BARRIER KINETICS
JAY MCLAREN
- 18:00 FLUID FLOW FROM CILIARY CAPILLARIES TO ANTERIOR CHAMBER: A PARALLEL PATHWAY CONTRIBUTING TO AQUEOUS HUMOR DYNAMICS
OSCAR CANDIA, Rosana Gerometta



SAN FRANCISCO **ISER**
XXI BIENNIAL MEETING
HYATT REGENCY SAN FRANCISCO AT EMBARCADERO
JULY 20-24, 2014

Schedule – Posters

SCHEDULE – POSTERS



International Society
for Eye Research

Anterior Segment

Viewing: 10:00 – 10:30, 12:00 – 13:00 Session with Authors: 15:00 – 16:30

BOARD 1	RNA SEQUENCING-BASED COMPARATIVE TRANSCRIPTOME ANALYSIS OF BALB/C AND C57BL/6 MOUSE CORNEAS OF NEOVASCULARIZATION GUANGYU LI, Don Yuen, Lu Chen
BOARD 2	INFLUENCE OF MORPHOLOGIC ALTERATIONS OF DESCEMET'S MEMBRANE ON THE CORNEAL EDEMA IN FUCHS' ENDOTHELIAL DYSTROPHY TOBIAS BROCKMANN, Claudia Brockmann, Anna-Karina Maier, Enken Gundlach, Eckart Bertelmann, Antonia Joussem, Necip Torun
BOARD 3	EVALUATION OF A NOVEL ARTIFICIAL TEAR IN THE PREVENTION AND TREATMENT OF DRY EYE IN AN ANIMAL MODEL WEI CHEN, Yujing Zhe, Jinyang Li, Haixia Liu
BOARD 4	STABILITY OF A NOVEL BACTERIAL LIPASE INHIBITOR IN THE PRESENCE OF BACTERIA AND BASAL TEARS JUDITH FLANAGAN, Eric Papas, Neeta Khandekar
BOARD 5	PDGF-BB EFFECTS DURING HUMAN CORNEAL STROMA WOUND REPAIR IN VITRO PATRICIA GALLEGGO, Roberto Cantalapiedra-Rodriguez, Carmen Martínez-García, María Cruz Valsero, José Garrote, Lucía Ibares
BOARD 6	A NOVEL PRESSED POROUS SILICON-POLYCAPROLACTONE COMPOSITE AS A DUAL-PURPOSE OPHTHALMIC IMPLANT YAZAD IRANI, Sonja Klebe, Yuan Tian, Mengjia Wang, Jeffery Coffey, Keryn Williams, Nicolas Voelcker
BOARD 7	PREPARATION OF OPHTHALMIC FORMULATIONS CONTAINING ITS NANOPARTICLES YOSHIMASA ITO, Norio Okamoto, Yoshikazu Shimomura, Noriaki Nagai, Chiaki Yoshioka
BOARD 8	OCULAR PHARMACOKINETICS COMPARISON BETWEEN PATADAY® VERSUS 0.77% NEWLY DEVELOPED OLOPATADINE TO MALE NZW RABBITS GANESH IYER, Jaime Yanez, Scott Womble, James Chastain
BOARD 9	KERATOCONUS: ROLE OF THE TGF-β SIGNALING PATHWAY DIMITRIOS KARAMICHOS, Hui Qi, Shrestha Priyadarsini, Akhee Sarker-Nag, Nawajes Mandal
BOARD 10	HUMAN CORNEAL STROMA WOUND REPAIR IN VITRO AFTER BFGF TREATMENT CARMEN MARTINEZ-GARCIA, Roberto Cantalapiedra-Rodriguez, Patricia Gallego-Muñoz, María Cruz Valsero, José Garrote, Lucía Ibares-Frías
BOARD 11	CONDITIONAL DELETION OF AP-2β IN NEURAL CREST CELL POPULATIONS RESULTS IN DYSGENESIS OF STRUCTURES IN THE ANTERIOR SEGMENT OF THE EYE VANESSA MARTINO, Judith West-Mays, Mizna Zaveri, Trevor Williams
BOARD 12	THE HUMAN TEAR LIPIDOME: DAY-TO-DAY VARIATION AND THE DIFFERENCES BETWEEN INDIVIDUALS TODD MITCHELL, Kunnen Carolina, Eric Papas, Percy Lazon de la Jara, Simon Brown, Mark Willcox, Stephen Blanksby
BOARD 13	EFFECTS OF LOSS OF TRPM2 ON THE INFLAMMATION AND SCARRING AFTER AN ALKALI-BURNED CORNEA IN MICE YUKA OKADA, Masayasu Miyajima, Kumi Shirai, Shizuya Saika
BOARD 14	INHIBITION OF LYMPHANGIOGENESIS AND HEMANGIOGENESIS IN CORNEAL INFLAMMATION BY SUBCONJUNCTIVAL PROX1 siRNA INJECTION IN RATS CHANG RAE RHO, Kyung-Sun Na, Kyung Jin Cho
BOARD 15	HISTOLOGICAL EXAMINATION OF THE CORNEAL FILAMENT IN FILAMENTARY KERATITIS HIDETOSHI TANIOKA, Takaharu Mochizuki, Takashi Yamanouchi, Osamu Katsuta, Kouichi Kawazu

BOARD 16	DISTRIBUTION OF GLYCOSYLATED PROTEINS ON THE SURFACE OF CORNEAL EPITHELIAL CELL CULTURES BERNARDO YANEZ SOTO, Nicholas Abbott, Vijay Ragunathan, Christopher Murphy, Brian Leonard
BOARD 17	THREE NOVEL MUTATIONS, P24R, G31R AND G463R IN COL8A2 GENE OF KOREAN PATIENTS WITH FUCHS' CORNEA DYSTROPHY YOUNG-SIK YOO, Jee-Won Mok, Woong-Joo Whang, Sung-A Lim, Choun-Ki Joo
BOARD 18	COMPARISON OF ENDOTOXIN-INDUCED UVEITIS MODEL AND EXPERIMENTAL AUTOIMMUNE UVEITIS MODEL IN LEWIS RATS FOR DRUG SCREENING LICHUN ZHONG, Laxman Desai
BOARD 19	PHO2SPHO-CORTACTIN AND CAVEOLIN COLOCALIZE TO PILS THAT ARE SITES OF ECM DEGRADATION MINI AGA, John Bradley, Ted Acott
BOARD 20	ON THE SEARCH FOR ADULT STEM CELLS IN THE TRABECULAR MESHWORK OUTFLOW PATHWAY OF THE PRIMATE EYE BARBARA BRAUNGER, B'Ann Gabelt, Julie Kiland, Elizabeth Hennes-Beann, Paul Kaufman, Bahar Ademoglu, Ernst Tamm, Kevin Brunner
BOARD 21	INVESTIGATION OF ENUCLEATED MOUSE EYES IN ORGAN CULTURE JASON CHANG, Darryl R. Overby, W. Michael Dismuke, W. Daniel Stamer
BOARD 22	PRESERVATION OF RETINAL GANGLION CELL FUNCTION BY TAUROURSODEOXYCHOLIC ACID NICOLAS CUENCA, Mercedes Palmero, Laura Fernandez-Sanchez, Pedro Lax, Gema Esquiva, Violeta Gomez-Vicente, Francisco Germain, Pedro De la Villa
BOARD 23	CRYSTAL STRUCTURE OF THE OLFACTOMEDIN DOMAIN OF MYOCILIN REBECCA DONEGAN, Raquel Lieberman
BOARD 24	SPHINGOLIPIDS AND CERAMIDES OF MOUSE AQUEOUS HUMOR: COMPARATIVE PROFILES FROM NORMOTENSIVE AND HYPERTENSIVE DBA/2J MICE GENEA EDWARDS, Katyayini Aribindi, Yenifer Guerra, Sanjoy Bhattacharya
BOARD 25	INVESTIGATING THE INFLUENCE OF BLAST ON CELLULARITY IN THE RETINAL GANGLION CELL LAYER IN A MOUSE MODEL OF BLAST-INDUCED TRAUMATIC BRAIN INJURY USING A SEMI-AUTOMATED TECHNIQUE ADAM HEDBERG-BUENZ, Matt Harper, Michael Anderson
BOARD 26	TRANSCRIPTOME ANALYSIS OF LASER-CAPTURED RETINAL GANGLION CELL LAYER REVEALS ROBUST EXPRESSION AND ENRICHMENT OF CRYSTALLIN TRANSCRIPTS STEVE T. HUYNH, Deborah Otteson
BOARD 27	EFFECT OF IMMUNIZATION WITH OCULAR ANTIGENS ON EXTRACELLULAR MATRIX AND GLIAL CELLS STEPHANIE JOACHIM, Jacqueline Reinhard, Susanne Wiemann, Andreas Faissner, Sabrina Reinehr, Sandra Kuehn, Rozina Noristani, Burkhard Dick
BOARD 28	GAP JUNCTIONS AID DIFFERENTIATION OF INDUCED PLURIPOTENT STEM CELLS INTO TRABECULAR MESHWORK-LIKE CELLS MARY KELLEY, Xinbo Li, Ted Acott
BOARD 29	EFFECTIVE AND PROSPECTIVENESS OF SELECTIVE LASER TRABECULOPLASTY IN TREATMENT OF PRIMARY OPEN ANGLE GLAUCOMA PATIENTS GLEB KRISHTOPENKO, Nikolai Pozniak, Sergei Pozniak, Nikolai Kovshel
BOARD 30	PRIMARY OPEN ANGLE GLAUCOMA AND PHACOEMULSIFICATION IRINA KUDERKO, Nikolai Pozniak, Sergei Pozniak, Nikolai Kovshel, Gleb Krishtopenko, Pavel Beliakovskii
BOARD 31	DIFFERENCE IN RELATIONSHIP BETWEEN CHOROIDAL THICKNESS MEASURED BY CIRRUS OCT AND HEMODYNAMIC PARAMETERS AMONG NORMAL SUBJECTS AND NORMAL TENSION GLAUCOMA PATIENTS MARVIN LEE, Jaehong Ahn
BOARD 32	FUNCTIONAL CELLULAR CONSEQUENCES OF AGEING IN PORCINE ANGULAR AQUEOUS PLEXI YUAN LEI, William Stamer, Xinghuai Sun, Jihong Wu
BOARD 33	TIGHT JUNCTION PROTEIN CLAUDIN-1 IS A MARKER FOR SCHLEMM'S CANAL CELLS XINBO LI, Ted Acott, Mary Kelley

BOARD 34	A CASE OF BENIGN NMO SPECTRUM DISORDER DURING PREGNANCY RYUTARO AKIBA, Toshiyuki Oshitari, Shuichi Yamamoto, Hirotaka Yokouchi, Yuuta Kitamura
BOARD 35	THE V-DOMAIN IG SUPPRESSOR OF CELL ACTIVATION (VISTA) PLAYS AN ESSENTIAL ROLE IN THE ACCEPTANCE OF CORNEAL ALLOGRAFTS TOMOYUKI KUNISHIGE, Hiroko Taniguchi, Tatsukuni Ohno, Miyuki Azuma, Junko Hori
BOARD 36	REGULATION OF THE MICROGLIAL PHAGOCYTOSIS-SENSOR TREM2 (CHR6P21) BY AN NF-KB-SENSITIVE MIRNA-34A (CHR 1P36) IN AGE-RELATED MACULAR DEGENERATION (AMD) WALTER LUKIW, Perna Dua, James Hill, Peter Alexandrov, Surjyadipta Bhattacharjee, Brandon Jones, Yuhai Zhao
BOARD 37	THE POSSIBLE ROLE OF BRADYKININ IN OCULAR INFLAMMATION: UVEITIC MACULAR OEDEMA AS AN EXAMPLE KELLY MAI, Arushi Singh, Denis Wakefield, Helder Marcal, Peter McCluskey
BOARD 38	HERPES SIMPLEX VIRUS-1 STRAIN KOS INDUCES CLINICAL AND IMMUNOLOGICAL CHANGES IN ACUTE HERPETIC KERATITIS HAMIDREZA MOEIN, Ahmad kheirkhah, Deshea Harris, Pedram Hamrah
BOARD 39	LONG-TERM OBSERVATION OF MURINE MODELS OF ANTERIOR SCLERITIS HIROKO TANIGUCHI, Yuki Kitahara, Junko Hori
BOARD 40	ADAMTS9 AS A CANDIDATE GENE IN PATHOGENESIS OF ANTERIOR SEGMENT DYSGENESIS JOHANNE DUBAIL, Stephanie Hagstrom, Elias Traboulsi, Michael Jenkins, Suneel Apte
BOARD 41	NANOSCALE TOPOGRAPHY AFFECTS TGFβ-INDUCED EMT OF LENS EPITHELIAL CELLS SCOTT BOWMAN, Paul Russell, Vijay Raghunathan, Chris Murphy, Judith West-Mays
BOARD 42	A ROLE FOR HEDGEHOG SIGNALING DURING LENS AND CORNEAL DEVELOPMENT ROBB DE IONGH, Janet Choi, Chaotung Ting, Lidia Trogrlic, Stefan Milevski, Gemma Martinez, Mary Familiarì
BOARD 43	SEQUENCE – STRUCTURE – FUNCTION OF LENS CRYSTALLINS STÉPHANIE FINET, Fériel Skouri-Panet, Céline Férard, Elodie Duprat
BOARD 44	CHARACTERIZATION OF THE CHILDHOOD LAMELLAR CATARACT IN TRANSGENIC MICE: IMPAIRMENT OF SECONDARY FIBER CELLS MORPHOGENESIS RAJENDRA GANGALUM, Zhe Jing, Ankur Bhat, Yoshiko Nagaoka, Sophie Deng, Meisheng Jiang, Suraj Bhat
BOARD 45	DNMT1 EXPRESSION IS REQUIRED FOR LENS EPITHELIAL CELL SURVIVAL EVAN HOROWITZ, Thanh Hoang, Blake Rasor, Blake Chaffee, Michael Robinson
BOARD 46	LECS FROM MMP-9 KO MICE SHOW RESISTANCE TO TGFβ-INDUCED EMT ANNA KOROL, Judith West-Mays
BOARD 47	UV-TREATMENT OF THE RAT EYE INCREASES CRYSTALLIN EXPRESSION IN THE RETINA KIRSTEN LAMPI, Emily Ho
BOARD 48	ALPHA A-CRYSTALLIN PREVENTS LENS EPITHELIAL CELL APOPTOSIS THROUGH NEGATIVE REGULATION OF P53-MEDIATED SIGNALING PATHWAY DAVID LI
BOARD 49	CHARACTERIZATION OF THE V41M MUTANT OF HUMAN GAMMA S CRYSTALLIN – HOW IS IT CATARACTOGENIC? AJAY PANDE, Jayanti Pande
BOARD 50	THE OCULAR LENS AND ENVIRONMENTAL INSULT: INTERESTING NOVEL OBSERVATIONS SHIWANI SHARMA, Maurizio Ronci, Nicolas Voelcker, Mark Corbett, Sarah Martin, Alpna Dave, Kathryn Burdon, Jamie Craig
BOARD 51	PENETRATION OF TOPICALLY APPLIED CAFFEINE TO THE LENS AND BLOOD CIRCULATION PER SÖDERBERG, Erik Forsman, Jonas Bergquist, Zhaohua Yu, Nooshin Talebizadeh, Martin Kronschräger, Stefan Löfgren
BOARD 52	LENS STIFFNESS IN CONNEXIN MUTANT LENSES WIKTOR STOPKA, Eddie Wang, Tom Libby, Chun-Hong Xia, Xiaohua Gong, Hong Ma

BOARD 53	CASPASE-3 IN ULTRAVIOLET RADIATION CATARACT NOOSHIN TALEBIZADEH, Zhaohua Yu, Martin Kronschräger, Per Söderberg
BOARD 54	CONTROL OF LENS FIBER DEVELOPMENT BY LHX2-REGULATED NEURORETINAL FGFS THUZAR THEIN, Jimmy de Melo, Cristina Zibetti, Seth Blackshaw
BOARD 55	STATISTICAL-THERMODYNAMIC MODEL FOR NONMONOTONIC DEPENDENCE OF LENS PROTEIN LIGHT SCATTERING AND PHASE BOUNDARIES ON MOLECULAR INTERACTIONS GEORGE THURSTON, David Ross
BOARD 56	MODULATION OF THE PHOSPHORYLATION STATUS OF THE NA⁺-K⁺-2CL⁻ COTRANSPORTER IN THE BOVINE LENS IRENE VORONTSOVA, Paul Donaldson, Julie Lim
BOARD 57	ESTABLISHING A MODEL TO INVESTIGATE ANTERIOR EPITHELIAL CELL DIVISION IN WHOLE PIG LENSES REBECCA ZOLTOSKI, Taia Cordel, Richard Miller, Steven Quan, Andrew Ritter, George McArdle

Tuesday, 22 July 2014 POSTER SESSION 2

Posterior Segment

Viewing: 10:00 – 10:30, 11:45 – 13:00 Session with Authors: 15:00 – 16:30

BOARD 1	SIMVASTATINS INHIBIT PATHOLOGICAL RETINAL ANGIOGENESIS IN VLDLR MOUSE MODEL SABU ABRAHAM, John Greenwood, Steven Moss, Xiaomeng Wang
BOARD 2	INTERACTIVE REGULATORY NETWORKS IN RETINAL ISCHEMIA-REPERFUSION INJURY KALINA ANDREEVA, Maha Soliman, Nigel Cooper
BOARD 3	EXPRESSION OF INTERCELLULAR ADHESION MOLECULE-1 BY HUMAN RETINAL ENDOTHELIAL CELLS IN RESPONSE TO INFLAMMATORY STIMULI BINOY APPUKUTTAN, Liam Ashander, Justine Smith
BOARD 4	PGRMC1, ALSO KNOWN AS SIGMA RECEPTOR 2, IS A CRITICAL REGULATOR OF IRON HOMEOSTASIS IN THE RETINA PACHIAPPAN ARJUNAN, Jaya Gnana-Prakasam, Sudha Ananth, Ganapathy Vadivel, Sylvia Smith
BOARD 5	CELLULAR THERAPY WITH KAINATIS OPTICONEUROPATHY PAVEL BELIAKOUSKI, Nikolai Pozniak, Sergei Pozniak, Irina Kuderko
BOARD 6	RETINAL UPREGULATION OF PRO-INFLAMMATORY MEDIATORS AND GROWTH FACTORS IN HIGH FAT DIET FED TYPE 2 DIABETIC MICE MENAKSHI BHAT, Souhad Akoum, Rejean Couture, Elvire Vaucher
BOARD 7	NEURITE REGENERATION IN ADULT RAT RETINAS EXPOSED TO LOW DOSE ADVANCED GLYCATION END-PRODUCTS AND REGENERATIVE EFFECTS OF NEUROTROPHIN-4 GUZEL BIKBOVA, Toshiyuki Oshitari, Shuichi Yamamoto
BOARD 8	DETECTION AND LOCALIZATION OF MICRORNA-34A BY IN SITU HYBRIDIZATION IN THE POSTERIOR POLE OF THE MOUSE EYE MATTHEW BORDBARI, Zeljka Smit-Mcbride, Krisztina Forward, Anthony Nguyen, Leonard Hjelmeland
BOARD 9	NFATC1 AND NFATC2 DIFFERENTIALLY REGULATE TNFα- AND VEGF-INDUCED INFLAMMATION IN RETINAL MICROVASCULAR ENDOTHELIAL CELLS COLIN BRETZ, John Penn
BOARD 10	THE BERLIN FAT MOUSE - A NEW MODEL TO INVESTIGATE RETINAL DEGENERATION CLAUDIA BROCKMANN, Tobias Brockmann, Sabrina Dege, Sergej Skosyrski, Olaf Strauss, Antonia Jousen
BOARD 11	PROMOTING BIOAVAILABILITY OF EPOXYEICOSATRIENOIC ACID INHIBITS TNFα-INDUCED RETINAL VASCULAR INFLAMMATION MEGAN CAPOZZI, John Penn

BOARD 12	DAYLIGHT VISION REPAIR BY CELL TRANSPLANTATION TIAGO FERREIRA, Kai Postel, Thomas Kurth, Marius Ader, Henrike Stutzki, Günther Zeck
BOARD 13	EFFECT OF GLUCOCORTICOIDS ON NEURONAL AND VASCULAR PATHOLOGY IN A TRANSGENIC MODEL OF SELECTIVE MÜLLER CELL ABLATION MARK GILLIES, Weiyong Shen, So Ra Lee, Joana Araujo, Sook Chung, Ling Zhu
BOARD 14	ANTI-ANGIOGENIC EFFECT OF BAICALIN IN A MOUSE MODEL OF OXYGEN-INDUCED RETINOPATHY JAE WOOK HAN, Su Ah Kim, Hye Bin Yim, KuiDong Kang
BOARD 15	INHIBITION OF $\alpha\beta$ CRYSTALLIN INDUCED MESENCHYMAL TO EPITHELIAL TRANSITION IN RPE CELLS KEIJIRO ISHIKAWA, Christine Spee, David Hinton, Ram Kannan, Sreekumar Parameswaran
BOARD 16	RETBINDIN IS A NOVEL PHOTORECEPTOR-SPECIFIC PROTEIN AND A MEMBER OF THE INTER-PHOTORECEPTOR MATRIX RYAN KELLEY, Muna Naash, Muayyad Al-Ubaidi
BOARD 17	GENOTYPE-PHENOTYPE ANALYSIS IN PATIENTS WITH AUTOSOMAL RECESSIVE RETINITIS PIGMENTOSA DUE TO PDE6A MUTATIONS DITTA ZOBOR, Gergely Zobor, Nicole Weisschuh, Bernd Wissinger, Susanne Kohl, Eberhart Zrenner
BOARD 18	ALDOSE REDUCTASE DEFICIENCY PROTECTS THE NEONATAL MOUSE RETINA AGAINST OXYGEN-INDUCED RETINOPATHY AMY LO, Sookja Chung, Zhongjie Fu, Shen Nian
BOARD 19	DERIVATION AND DISEASE MODELING OF HUMAN PLURIPOTENT STEM CELL-DERIVED RETINAL GANGLION CELLS JASON MEYER, Sarah Ohlemacher
BOARD 20	MOLECULAR CHARACTERIZATION OF NOVEL COMPOUND HETEROZYGOUS MUTANTS ASSOCIATED WITH LEBER CONGENITAL AMAUROSIS IN CHINESE FAMILY YURIKO MINEGISHI, Xunlun Sheng, Takeshi Iwata
BOARD 21	TIME COURSE OF ACTIVATION AND RECOVERY FROM ADAPTATION TO A BACKGROUND LIGHT IN MOUSE RODS ALA MORSHEDIAN
BOARD 22	WNT/B-CATENIN SIGNALING IN MICROVASCULAR ENDOTHELIAL AND MÜLLER CELLS IS ESSENTIAL FOR RETINAL VASCULAR DEVELOPMENT AND REPAIR BIRGIT MÜLLER, Daniel Wöhl, Ernst Tamm, Andreas Ohlmann
BOARD 23	EXOGENOUS $\alpha\beta$-CRYSTALLIN PROMOTES TUBULOGENESIS IN HUMAN RETINAL ENDOTHELIAL CELLS ROOBAN NAHOMI, Scott Howell, Ram Nagaraj
BOARD 24	DIFFERENTIAL EXPRESSION OF MIRNAS IN ROD PHOTORECEPTORS AND MÜLLER GLIA HEBERTO QUINTERO, Monica Lamas
BOARD 25	DTGR: A NEW RAT MODEL FOR SYSTEMIC HYPERTENSIVE RETINOPATHY NADINE REICHART, Christina Herrspiegel, Sergej Skosyrski, Nadine Haase, Ralf Dechend, Olaf Strauss
BOARD 26	MICRO RNAS ASSOCIATED WITH A MODEL OF RETINAL DEGENERATION KARTIK SAXENA, Riccardo Natoli, Jan Provis, Matt Rutar
BOARD 27	IMMUNOPROTEASOME ROLE IN STRESS RESPONSE AND TEMPORAL PTEN/AKT SIGNALING AFTER OPTIC NERVE CRUSH (ONC) NATHAN SCHULD, Stacy Hussong, Rebecca Kapphahn, Abrar Rageh, Neal Heuss, Dale Gregerson, Deborah Ferrington
BOARD 28	REPAIR OF RHODOPSIN MRNA BY SPLICEOSOME-MEDIATED RNA TRANS-SPLICING: A NEW APPROACH FOR AUTOSOMAL DOMINANT RETINITIS PIGMENTOSA ADELINE BERGER, Stéphanie Lorain, Charlene Josephine, Mélissa Desrosiers, Cécile Peccate, Thomas Voit, Luis Garcia, José-Alain Sahel, Alexis-Pierre Bemelmans
BOARD 29	DIFFERENTIATION OF 3D RETINA-LIKE STRUCTURES FROM PLURIPOTENT STEM CELLS MATTHEW SMART, Conor Ramsden, Michael Powner, Peter Coffey, Lyndon Da Cruz, Li Li Chen

BOARD 30	RDS GLYCOSYLATION; A WINDOW INTO THE DIFFERENTIAL ROLE OF RDS IN RODS AND CONES MICHAEL STUCK, Shannon Conley, Muna Naash
BOARD 31	THE ROLE OF GAPDH/SIAH1 IN HUMAN RETINAL PERICYTE APOPTOSIS SANDRA SUAREZ, Ashwath Jayagopal, John Penn
BOARD 32	NEURONAL VHL DELETION INDUCES PERSISTENT FETAL VASCULATURE AND STRONG SUPPRESSION OF RETINAL VASCULAR FORMATION YOSHIHIKO USUI, Toshihide Kurihara, Edith Aguilar, Carli Wittgrove, Daniel Feitelberg, Peter Westenskow, Martin Friedlander
BOARD 33	PHOTORECEPTOR REGENERATION IN TRANSGENIC MICE SHU-ZHEN WANG
BOARD 34	COMPLEMENT DEPOSITION IN THE RETINA OF RPE-SPECIFIC CFH-/- AND CFH-/-CFB-/- DOUBLE KNOCK-OUT MICE JENNIFER WILLIAMS, John Greenwood, Stephen Moss, Peter Adamson, Judy Latcham
BOARD 35	REVERSAL OF OXIDATIVE STRESS IN A MOUSE MODEL OF DRY AMD MANAS BISWAL, Yun Le, Zhaoyang Wang, Haoyu Mao, Hong Li, Alfred Lewin
BOARD 36	THE ZEBRAFISH AS A MODEL SYSTEM FOR THE ANALYSIS OF MELANOSOME BIOGENESIS IN THE RPE IN HEALTH AND HUMAN DISEASE THOMAS BURGOYNE, Marie O'Connor, Miguel Seabra, Daniel Cutler, Clare Futter
BOARD 37	CIGARETTE SMOKE AND NRF2 DEFICIENCY IMPAIRS MITOCHONDRIAL FUNCTION IN RETINAL PIGMENT EPITHELIAL (RPE) CELLS MARISOL CANO, Brad Barnett, Lei Wang, Sonny Dike, James Handa
BOARD 38	GENERATION OF IPS-RPE FROM PATIENTS WITH AUTOSOMAL DOMINANT VITREORETINOCHOROIDOPATHY AMANDA-JAYNE CARR, Britta Nommiste, Peter Coffey
BOARD 39	HUMAN ORGANIC ANION TRANSPORTING POLYPEPTIDE 1A2 (OATP1A2) IS A NOVEL MEDIATOR OF CELLULAR UPTAKE OF ALL-TRANS-RETINOL IN HUMAN RPE CELLS TING CHAN, Fanfan Zhou, Ke Wang, Ling Zhu, Weiyong Shen, Mark Gillies, Michele Madigan
BOARD 40	MAJOR AMERICAN DIETARY PATTERNS ARE RELATED TO RISK OF AGE-RELATED MACULAR DEGENERATION CHUNG-JUNG CHIU, Gary Gensler, Tricia Li, Fang Fang Zhang, Min-Lee Chang, Molly Schleicher, Allen Taylor
BOARD 41	A QUANTITATIVE MODEL OF CHOROIDAL NEOVASCULARIZATION IN THE RABBIT WILLIAM CULP, Jacklyn Salmon, Brian Gilger
BOARD 42	CHARACTERIZATION OF BEST1 ISOFORMS AND MIRNA LEVELS BETWEEN THE HUMAN MACULAR AND EXTRAMACULAR RETINAL PIGMENT EPITHELIUM SHEMIN ZENG, Arlene Drack, Budd Tucker, Edwin Stone, Robert Mullins
BOARD 43	A2E AND OTHER BIS-RETINOIDS: POTENTIAL INVOLVEMENT IN RPE PATHOBIOLOGY IN A RAT MODEL OF A HUMAN HEREDITARY CHOLESTEROL BIOSYNTHETIC DISORDER STEVEN FLIESLER, Bruce Pfeffer, Christopher Goulah, Keiko Ueda, Janet Sparrow
BOARD 44	ASSESSMENT OF CALCIPOTRIOL, VITAMIN D3 AND CHOLESTEROL AS NOVEL REAGENTS IN THE REPIGMENTATION OF ARPE-19 CELLS EVA GRINDLEY, Carl Sheridan, Rachel Williams
BOARD 45	MICRORNA-21 REGULATES PRORENIN RECEPTOR (PRR)-INDUCED VEGF SYNTHESIS THROUGH MODULATING MEK/ERK PATHWAY IN ARPE-19 CELLS DURING HYPERGLYCEMIA RASHIDUL HAQUE, P. Michael Iuvone, Kimberly Su Chung Choi, Daniel Park, Ashley Ngo, Samantha Gokhale, Jennifer Chung Ah Ro
BOARD 46	HISTOPATHOLOGY OF EYES FROM PATIENTS WITH AUTOSOMAL RECESSIVE RETINITIS PIGMENTOSA CAUSED BY NOVEL EYS MUTATIONS JOE HOLLYFIELD, John Chiang, Mary Rayborn, Vera Bonilha, Brent Bell, Meghan Marino, Gayle Pauer, Craig Beight, Elias Traboulsi, Stephanie Hagstrom

BOARD 47	AGING ALTERS THE PROTEOLYTIC ACTIVITY OF HUMAN RETINAL PIGMENT EPITHELIUM: IMPLICATIONS FOR THE PATHOGENESIS OF AGE-RELATED MACULAR DEGENERATION PAUL KAY, Umar Sharif, Luminita Paraoan, Yit Yang
BOARD 48	IRON IS REQUIRED FOR MATURATION OF AMYLOID PRECURSOR PROTEIN IN RETINAL PIGMENTED EPITHELIAL CELLS M. CHRISTINE MCGAHAN, Jill Harned, Marilyn Lall
BOARD 49	NOVEL PENTABLOCK COPOLYMER BASED NANOFORMULATIONS FOR SUSTAINED OCULAR DELIVERY OF PROTEIN THERAPEUTICS ASHIM MITRA
BOARD 50	HYPOXIA AND RETROMER AS POTENTIAL REGULATORS OF POLARIZED AMYLOID PRECURSOR PROTEIN EXPRESSION IN RPE CELLS PHILIP MZYK, Marilyn Lall, Steven Nagar, Mary McGahan
BOARD 51	VISUALIZATION OF IRON REGULATED CHANGES IN FERROPORTIN EXPRESSION AND SUBCELLULAR LOCALIZATION IN CULTURED CANINE RPE CELLS STEVEN NAGAR, Mary McGahan
BOARD 52	OVEREXPRESSION OF HTRA1 AND EXPOSURE TO CIGARETTE SMOKE EVOKE CHOROIDAL NEOVASCULARIZATION AND RETINAL DEPOSITS IN AGED MICE MAO NAKAYAMA, Daisuke Iejima, Masakazu Akahori, Junzo Kamei, Asako Goto, Takeshi Iwata
BOARD 53	A CELLULAR DISEASE MODEL SYSTEM FOR AUTOSOMAL-RECESSIVE BESTROPHINOPATHY: THE CREATION OF IPS-RPE FROM A PATIENT WITH PREMATURE STOP MUTATION (P.R200X) BRITTA NOMMISTE, Amanda Carr, Pete Coffey
BOARD 54	NUMBER AND LOCATION OF LESIONS FOR THE OPTIMIZATION OF THE EXAMINATION INTERVAL IN DIABETIC RETINOPATHY GIOVANNI OMETTO, Mogens Erlandsen, Toke Bek
BOARD 55	FETAL HEMOGLOBIN INDUCTION BY MONOMETHYLFUMARATE: RELEVANCE TO PREVENTION AND TREATMENT OF RETINOPATHY IN SICKLE CELL DISEASE (SCD) WANWISA PROMSOTE, Rajalakshmi Veeranan Karmegam, Vadivel Ganapathy, Pamela Martin, Sylvia Smith, Levi Makala, Biaoru Li, Betty Pace
BOARD 56	HISTOPATHOLOGY OF THE RETINA IN EYES FROM PATIENTS WITH BEST DISEASE CAUSED BY VMD2 MUTATIONS MARY RAYBORN, Vera Bonilha, Brent Bell, Meghan Marino, Gayle Pauer, Craig Beight, Elias Traboulsi, Stephanie Hasgstrom, Gerald Fishman, Joe Hollyfield
BOARD 57	A COMPARISON OF PHAGOCYTOSIS IN FETAL VS. STEM CELL DERIVED RPE CELLS DAVID ROUTMAN, David Hinton, Christine Spee, Danhong Zhu
BOARD 58	DIFFERENTIALLY EXPRESSED EXTRACELLULAR RNA IN SERUM OF AMD PATIENTS ZELJKA SMIT-MCBRIDE, Amar Patel, Anthony Nguyen, Matthew Bordbari, David Telander, Lawrence Morse
BOARD 59	A QUANTITATIVE STRUCTURAL COMPARISON OF THE MOUSE CENTRAL RETINA AND THE HUMAN MACULA: IS THE MOUSE AN APPROPRIATE MODEL FOR MACULAR DEGENERATION? STEFANIE VOLLAND, Julian Esteve-Rudd, David S. Williams
BOARD 60	GENE PROFILING OF POSTNATAL MFRPRD6 MUTANT EYES REVEALS DIFFERENTIAL ACCUMULATION OF PRSS56 MRNA PATSY NISHINA, Ramani Soundararajan, Jungyeon Won, Timothy Stearns, Wanda Hicks, Juergen Naggert, Mark Krebs
BOARD 61	RECOVERY OF TRANSEPITHELIAL ELECTRIC RESISTANCE FROM OXIDATIVE INJURY BY HUMANIN AND ROLE OF MITOCHONDRIA IN PROTECTION OF RPE CELLS SREEKUMAR PARAMESWARAN, Keijiro Ishikawa, Chris Spee, Hemal Mehta, Kelvin Yen, Pinchas Cohen, Ram Kannan, David Hinton
BOARD 62	CONNECTIVE TISSUE GROWTH FACTOR IS A KEY REGULATOR OF OXYGEN-INDUCED RETINOPATHY BRAHIM CHAQOUR, Maria Grant, Haibo Liu, Rahul Parmar, Monika Kamalska, Yoon Ji Kim, David Lovett, Hembindu Chintala

Anterior/Posterior Segment

Wednesday Viewing: 10:00 – 10:30, 12:00 – 13:00

Thursday Viewing: 10:00 – 10:30, 11:45 – 13:00 Session with Authors: 15:00 – 16:30

BOARD 1	FROM COLOBOMA TO CYCLOPIA: DECIPHERING THE MORPHOGENETIC ROLE OF THE NEURAL CREST ON EYE DEVELOPMENT SOUFIEN SGHARI, Sophie Creuzet
BOARD 2	REDUCED EFFECTIVE FILTRATION AREA FOR AQUEOUS OUTFLOW IS CORRELATED WITH DECREASED OUTFLOW FACILITY IN SPHINGOSINE-1-PHOSPHATE TREATED BOVINE EYES LAIYIN MA, Elliot Cha, Haiyan Gong
BOARD 3	BLOCKADE OF ADENOSINE A2A RECEPTOR PROTECTS RETINAL GANGLION CELLS AGAINST ELEVATED PRESSURE-INDUCED CELL DEATH BY CONTROLLING NEUROINFLAMMATION MARIA HELENA MADEIRA, Francisco Gonçalves, Rodrigo Cunha, António Ambrósio, Ana Santiago
BOARD 4	GENETICALLY DISSECTING THE PRIMARY SITE OF PATHOGENESIS IN COL4A1 MEDIATED ANTERIOR SEGMENT DYSGENESIS MAO MAO, Douglas Gould
BOARD 5	NORMAL AND GLAUCOMATOUS HUMAN LAMINA CRIBROSA AND TRABECULAR MESHWORK CELL BEHAVIOURS AS DETERMINED BY RIGIDITY OF THE SURROUNDING EXTRACELLULAR MATRIX SARA MCNALLY, Colm O'Brien
BOARD 6	SENSITIVITY OF THE <i>NEE</i> ALLELE TO GENETIC BACKGROUND KACIE MEYER, Michael Anderson
BOARD 7	NORRIN INHIBITS THE DEVELOPMENT OF GLAUCOMA IN DBA/2J MICE ANDREAS OHLMANN, Ludwig Zeilbeck, Stephanie Leopold, Ernst Tamm
BOARD 8	ADENOSINE A2A RECEPTOR BLOCKADE AND CAFFEINE MODULATE RETINAL NEUROINFLAMMATION FOLLOWING ISCHEMIA-REPERFUSION INJURY ANA RAQUEL SANTIAGO, Raquel Boia, Pedro Tralhão, Filipe Elvas, Maria Madeira, António Francisco Ambrósio
BOARD 9	CROSS-TALK BETWEEN AQUAPORINS AND INFLAMMATORY MEDIATORS IN GLAUCOMATOUS IRIS AND TRABECULAR MESHWORK RAJESH SASIKUMAR, Anuprita Ghosh, Arkashubra Ghosh, Reshma R, Rohit Shetty
BOARD 10	CONSEQUENCE OF ELEVATED IOP IN MMP-9 DEFICIENT MICE ON BRN3A+ RGC COUNT ANUJA SIWAKOTI, Judith West-Mays
BOARD 11	A GENOMIC LOCUS MODULATING GANGLION CELL DEATH FOLLOWING ELEVATED IOP IN THE MOUSE FELIX STRUEBING, Michael Hauser, Louis Pasquale, Janey Wiggs, Eldon Geisert, Justin Templeton, Xiangdi Wang
BOARD 12	CUMULATIVE MTDNA DAMAGE AND MUTATIONS CONTRIBUTE TO THE PROGRESSIVE LOSS OF RGCS IN A RAT MODEL OF GLAUCOMA JIHONG WU, Fengjuan Gao, Xinya Chen
BOARD 13	COMPARATIVE STUDIES OF THE ENDOTHELIAL GLYCOCALYX LAYER IN THE AQUEOUS OUTFLOW PATHWAY OF HUMAN AND BOVINE EYES CHEN YUAN YANG, Mark Johnson, Tiffany Huynh, Haiyan Gong
BOARD 14	A MICRORNA SIGNATURE OF RETINA IDENTIFIED BY DEEP SEQUENCING FOLLOWING CHRONIC OCULAR HYPERTENSION OF RAT SHENGHAI ZHANG, Xinghuai Sun, Feng Gao
BOARD 15	AN INVESTIGATION OF THE VARIATION IN VISUAL ACUITY THRESHOLDS MEASURED USING KAY PICTURE OPTOTYPES WHEN COMPARED TO A LANDOLT C TARGET ANDREW COLLINS, Nicola Anstice

BOARD 16	COMPLEMENT SYNTHESIS AND PROPAGATION BY IBA1+ MONOCYTES/MICROGLIA IN AMD AND IN ANIMAL MODELS OF AGING AND DEGENERATION MATTHEW RUTAR
BOARD 17	REDUCTION OF ACUTE POST-OPERATIVE INFLAMMATION BY TOPICAL RX-10045 IN THE RABBIT PARACENTESIS MODEL BRIAN GILGER, Jacklyn Salmon, Henry Goodell, Poonam Velagaleti, Sidney Weiss
BOARD 18	IN VIVO IMAGING OF MICROGLIA IN CHOROIDAL NEOVASCULARIZATION SERGIO CRESPO-GARCIA, Sergej Skosyrski, Anna Maier, Antonia Jousen, Olaf Strauss
BOARD 19	HIGH SPATIAL RESOLUTION IN VIVO MAGNETIC RESONANCE IMAGING OF THE HUMAN EYE, ORBIT, NERVUS OPTICUS AND OPTIC NERVE SHEATH AT 7.0 TESLA OLIVER STACHS, Jan Rieger, Max Muhle, Michael Schwerter, Thoralf Niendorf, Rudolf Guthoff, Paul Krueger, Soenke Langner, Tobias Lindner
BOARD 20	EFFECTS OF CYCLOHEXYLADENOSINE ON OUTFLOW FACILITY IN PERFUSED ENUCLEATED MOUSE EYES W. MICHAEL DISMUKE, Iris Navarro, W. Dan Stamer, Mortimer Civan
BOARD 21	SIGMA-1 RECEPTOR INCREASES MITOCHONDRIAL MEMBRANE POTENTIAL IN GLUCOSE AND OXYGEN DEPRIVED RETINAL GANGLION CELLS DORETTE ELLIS, Yong Park, Brett Mueller, Linya Li, Hai-Ying Ma, Thomas Yorio
BOARD 22	RETINAL WHITE BLOOD CELL FLUX AND SYSTEMIC BLOOD PRESSURE IN PATIENTS WITH TYPE 1 DIABETES GABRIELE FUCHSJÄGER-MAYRL, Stefan Palkovits, Leopold Schmetterer
BOARD 23	CX3CR1 DEFICIENCY EXACERBATES PHOTORECEPTOR TOXICITY OF MONONUCLEAR PHAGOCYTE IN A MODEL OF SUBRETINAL INFLAMMATION SHULONG HU, Bertrand Calippe, Sophie Lavalette, Florian Sennlaub, Xavier Guillonnet
BOARD 24	RESTORING LIGHT SENSITIVITY IN BLIND RETINAE USING THIRD GENERATION PHOTOPHARMACOLOGY LAURA LAPRELL, Martin Sumser, Dirk Trauner
BOARD 25	MYOPIA PROGRESSION IN YOUNG ADULTS YI PANG
BOARD 26	NMDA AND AMPA RECEPTOR STIMULATION IN RETINAL GANGLION CELLS INDUCES PROLONGED PHOSPHORYLATION OF CREB AND INCREASES RESISTANCE TO APOPTOSIS YONG PARK, Brett Mueller, Thomas Yorio
BOARD 27	PATIENT EXPERIENCE AND COMFORT DURING VITRECTOMY SURGERY WITH MONITORED ANESTHESIA CARE HEMA RAMKUMAR, Azadeh Khatibi, Isaac Ezon, Cheryl Arcinue, Giulio Barteselli, Joseph Nezgoda, William Freeman, Michael Goldbaum
BOARD 28	THE EFFECT OF DOPAMINE D1 AND D2 AGONISTS AND ANTAGONISTS ON THE DEVELOPMENT OF FORM DEPRIVATION MYOPIA IN TREE SHREWS ALEX WARD, John Siegwart, Michael Frost, Thomas Norton
BOARD 29	MODULATION OF PRO-INFLAMMATORY RESPONSES IN THE RETINA BY NEUROPEPTIDE Y: THE ROLE OF NPY Y1 RECEPTOR ANTÓNIO FRANCISCO AMBRÓSIO, Filipe Elvas, Sandra Correia, João Martins, Maria Madeira, Tiago Martins, Raquel Boia, Cláudia Cavadas, Ana Raquel Santiago
BOARD 30	A ROLE OF THE HOMEOPROTEIN TRANSCRIPTION FACTOR RAX IN POSTNATAL PHOTORECEPTOR DEVELOPMENT SHOICHI IRIE, Takahisa Furukawa
BOARD 31	SELECTIVE ABLATION OF VASCULATURE AFFECTS RETINAL NEUROGENESIS IN ZEBRAFISH EMBRYOS SUSOV DHAKAL, Deborah Stenkamp
BOARD 32	CHARACTERIZATION OF A BLIMP 1-SPECIFIC ENHANCER IN THE DEVELOPING RETINA JOSEPH BRZEZINSKI, Tatiana Eliseeva, Ko Uoon Park
BOARD 33	HYPERGLYCEMIA-DEPENDENT AND -INDEPENDENT ACTIVATION OF CASPASE-1 IN MÜLLER CELLS DERRICK FEENSTRA, Susanne Mohr

BOARD 34	FUNCTIONAL ROLES OF RAX HOMEOPROTEIN IN MOUSE RETINA DEVELOPMENT TAKAHISA FURUKAWA
BOARD 35	CONTRIBUTIONS OF VEGF AND PHOSPHORYLATION OF NITRIC OXIDE SYNTHASE IN THE DEVELOPMENT OF RETINOPATHY OF PREMATURITY ALIREZA HOSSEINI, Jorge Jacot, Frank Lattanzio, Nazita Yousefieh, Robin Looft-Wilson, Ryan Wade
BOARD 36	UNRAVELING THE THERAPEUTIC MECHANISMS OF HUMAN UMBILICAL CORD TISSUE-DERIVED CELLS (HUTC) IN RETINAL DEGENERATIVE DISEASES SEHWON KOH, Cagla Eroglu, Namsoo Kim, Henry Yin, Nadine Dejneka, Ian Harris
BOARD 37	THE ROLE OF THE ACTIN CYTOSKELETON AND RHO-ASSOCIATED COILED-COIL KINASES IN THE ADULT REGENERATING ZEBRAFISH RETINA MANUELA LAHNE, David Hyde
BOARD 38	HYPERGLYCEMIA INDUCES PYROPTOTIC CELL DEATH IN RETINAL MÜLLER CELLS SUSANNE MOHR
BOARD 39	REGENERATIVE EFFECT OF TAURINE-CONJUGATED URSODEOXYCHOLIC ACID AND NEUROTROPHIN-4 IN RAT RETINAS EXPOSED TO HIGH GLUCOSE TOSHIYUKI OSHITARI, Guzel Bikbova, Shuichi Yamamoto
BOARD 40	HIGH GLUCOSE ALTERS RETINAL ASTROCYTES PHENOTYPE THROUGH INCREASED PRODUCTION OF INFLAMMATORY CYTOKINES AND OXIDATIVE STRESS NADER SHEIBANI, Eui Seok Shin, Christine Sorenson
BOARD 41	GANGLION CELL LOSS IN RETINAL ISCHEMIA IS FACILITATED BY THE INFLAMMASOME VALERY SHESTOPALOV, Juan Pablo de Rivero Vaccari, Galina Dvorianchikova
BOARD 42	DISRUPTION OF NEURON LAMINATION IN THE MOUSE RETINA ALTERS DEVELOPMENT OF THE RETINAL VASCULATURE AARON SIMMONS, Peter Fuerst
BOARD 43	VISUAL PROPERTIES OF RCS RATS TRANSDUCED WITH MODIFIED VOLVOX CHANNELRHODOPSIN-1 ERIKO SUGANO, Fumiaki Nishiyama, Kitako Tabata, Namie Murayama, Maki Takahashi, Takehiko Saito, Hiroshi Tomita, Makoto Tamai
BOARD 44	DSCAM MEDIATES DENDRITE LAMINATION PATTERN IN THE MOUSE RETINA IN A DOSE DEPENDENT MANNER BY RESTRICTING THE STRATUM IN WHICH NEURITES ARBORIZE JOSHUA SUKEENA, Peter Fuerst, Ethan Hansen
BOARD 45	ANALYSIS OF ISOLATED ROD PHOTORECEPTORS FROM TRANSGENIC ZEBRAFISH CHI SUN, Deborah Stenkamp
BOARD 46	RETINAL DISTRIBUTION OF DISABLED-1 IN A DIURNAL MURINE RODENT, THE NILE GRASS RAT ARVICANTHIS NILOTICUS YVES SAUVÉ, Sharee Kuny, Frederic Gaillard
BOARD 47	INHIBITION OF THE TGFβ SIGNALING PATHWAY INDUCES OVERSHOOTING PROLIFERATION DURING RETINA REGENERATION IN ADULT ZEBRAFISH MARKUS TSCHOPP, Pauline Sallin, Anna Jazwinska, Christoph Tappeiner, Ellinor Maurer, Kaspar Schürch, Volker Enzmann
BOARD 48	WHOLE EXOME ANALYSIS IDENTIFIES FREQUENT CNGA1 MUTATIONS IN JAPANESE POPULATION WITH AUTOSOMAL RECESSIVE RETINITIS PIGMENTOSA SATOSHI KATAGIRI, Kazuki Kuniyoshi, Mineo Kondo, Shinji Ueno, Kazutoshi Yoshitake, Kazuho Ikeo, Masakazu Akahori, Kazushige Tsunoda, Takeshi Iwata, Yuri Sergeev, Kei Shinoda, Takaaki Hayashi, Hiroyuki Sasano, Yasuhiro Ohkuma, Hiroshi Tsuneoka, Masaaki Furuno ^o , Yohinori Tsurusaki, Naomichi Matsumoto
BOARD 49	HIGH FIDELITY HUMAN PLURIPOTENT STEM CELLS FOR OCULAR REGENERATIVE MEDICINE ELIAS ZAMBIDIS, Tea Soon Park, Jeffrey Huo, Ludovic Zimmerlin, Jasmin Agarwal, Imran Bhutto, Rhonda Grebes, Xiufeng Zhong, Christian Gutierrez, Valeria Canto-Soler, Gerard Luty
BOARD 50	AP-2A AND AP-2SS ARE REQUIRED FOR HORIZONTAL CELL DEVELOPMENT AND AMACRINE CELL MOSAIC PATTERNING MIZNA ZAVERI, Darshan Sapkota, Fuguo Wu, Xiuqian Mu, Alexander Ball, Judith West-Mays

SAN FRANCISCO **ISER**
XXI BIENNIAL MEETING
HYATT REGENCY SAN FRANCISCO AT EMBARCADERO
JULY 20–24, 2014

ABSTRACTS – ORAL PRESENTATIONS

Disclosures reflect financial relationships for presenting authors only.



International Society
for Eye Research

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The New Concept of the Blood–Aqueous Barrier	0424-0427
POSTER SESSION 1	NUMBERS
Anterior Segment	P101-P157
Cornea and Ocular Surface, Glaucoma, Ocular Immunology, Lens	
POSTER SESSION 2	NUMBERS
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Retinal Cell Biology, RPE Biology and Pathology	
POSTER SESSION 3	NUMBERS
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Retinal Neuroscience and Development, Glaucoma, Ocular Imaging, Physiology and Pharmacology	

LE01 – Crystallin Interactions in Health and Disease

O001

CRYSTALLIN INTERACTIONS IN HEALTH AND DISEASE

JAYANTI PANDE

DEPARTMENT OF CHEMISTRY, LIFE SCIENCES, UNIVERSITY AT ALBANY, SUNY

It is becoming increasingly evident that the crystallins, once believed to be confined to the lens fiber cells, are in fact expressed in a variety of cells beyond the lens, where they play significant functional roles. Optimal interactions of the crystallins with other members of the crystallin family have long been known to be critical for the establishment of short-range order in the fiber cell, and hence, to the maintenance of lens transparency. However, the manifestation of crystallin interactions beyond the lens, and the significance of such interactions in a number of pathologies, has only recently been recognized.

In this symposium, the importance and significance of the interactions of the small heat-shock proteins, alphaA- and alphaB- crystallins, in health and disease is highlighted by examining their structure, function, dynamics, chemical and genetic modifications, and self-assembly in the normal and pathological states within the lens and beyond, using a number of biophysical and biochemical techniques including high-resolution mass spectrometry and NMR spectroscopy. Altered interactions of alpha-, beta- and gamma-crystallins within normal and cataractous lenses with other partners that lead to post-translational modifications are also highlighted. The intriguing role of metal ions such as Cu⁺², implicated in several neurodegenerative diseases and cataract, in modulating the chaperone properties of the sHSPs, will be discussed. A novel development is the discovery of “peptide chaperones” — a variety of shorter peptides of alphaA-crystallin that can penetrate the cell membrane and exhibit cytoprotective properties.

IRB Status: Verified

Disclosures:

JAYANTI PANDE, PHD: No financial relationships to disclose

O002

DYNAMIC INTERACTIONS OF α B-CRYSTALLIN

JUSTIN BENESCH

UNIVERSITY OF OXFORD

We have developed and applied novel approaches, based primarily on mass spectrometry (MS), to overcome the challenges

posed by the inherent heterogeneity of sHSPs that allow the simultaneous gleanings of both structural and dynamical information on the sHSPs. Here we present detailed biophysical insights into the self-assembly of the polydisperse human sHSP α B-crystallin, and disease-related variants thereof. By combining MS with NMR spectroscopy, electron microscopy and X-ray crystallography we reveal both the likely oligomeric structures α B-crystallin adopts and the motions they undergo at equilibrium. We find that α B-crystallin is extremely dynamic, and uncover an allosteric coupling between interfaces in the protein. Our in vitro data suggest that it is these properties that are directly responsible for aberrant function in vivo.

IRB Status: None

Disclosures:

JUSTIN BENESCH, PHD: No financial relationships to disclose

O003

THE INTERACTION OF ALPHA-CRYSTALLIN WITH DISEASE-RELATED, AGGREGATING PROTEINS

JOHN CARVER

THE AUSTRALIAN NATIONAL UNIVERSITY

Alpha-crystallin, the principal lens protein, is composed of two closely related subunits alphaA and alphaB. Alpha-crystallin plays a dual and pivotal role in the lens: as a structural protein to ensure proper light refraction and as a molecular chaperone to prevent protein (crystallin) aggregation that may lead to cataract formation. The chaperone ability of alphaB-crystallin (and other small heat-shock proteins) has been utilised widely in tissues apart from the lens. Its elevated expression is associated with many diseases of protein misfolding and aggregation in addition to cataract, e.g. Alzheimer's, Parkinson's and Huntington's.

In this talk, I shall present some of our recent results investigating the structure and function of alphaB-crystallin, specifically its interactions with aggregating proteins including those associated with disease. The large size of the alphaB-crystallin oligomer along with its polydisperse and dynamic nature make studying the intact protein a challenge. To do so, requires a multi-faceted approach using a diversity of complementary spectroscopic, biophysical, protein chemical and cell biological techniques including NMR, CD and fluorescence spectroscopy, dynamic light scattering and electron microscopy. The results of this investigation provide insights into the mechanism of small heat-shock protein chaperone action in addition to potential avenues for the application of these proteins, and molecular chaperones in general, in the treatment of protein misfolding diseases, including cataract.

IRB Status: None

Disclosures:

JOHN CARVER, PHD: No financial relationships to disclose

FUNCTIONAL ELEMENTS IN HUMAN α B CRYSTALLIN

JOHN CLARK

UNIVERSITY OF WASHINGTON BIOLOGICAL STRUCTURE AND
OPHTHALMOLOGY

It is well established that cytoplasmic protein aggregation is closely linked with lens opacification in cataract formation, one of the best known disorders of human aging. The recent emphasis on protein – protein interactions in amyloid disease suggests similarities in regulatory mechanisms for cataract, neurodegenerative disease, sickle cell hemoglobin and biomedical problems involving diverse molecules that self-assemble to form aggregates and/or fibrils. Studies of small heat shock proteins (sHSP) determined their protective activity against some of the most important amyloid pathologies affecting human health. An endogenous regulator of protein aggregation, human α B crystallin, is the archetype for small stress proteins that participate in a variety of degenerative processes during aging. Cellular expression of α B crystallin increases in response to physical and chemical stress that include temperature, pH, hypoxia, osmotic pressure, proteolysis, chemical modification, oxidation, and changing metabolite levels which are characteristic of cardiovascular disease, cataract, and neurodegeneration. Multiple interactive amino acid sequences that map to the exposed surface of human α B crystallin can account for the diversity in the regulatory action of α B crystallin on the aggregation of unfolding proteins. The basis for the sensitivity of human α B crystallin to molecular destabilization during protein unfolding remains a key to the mechanism of sHSP regulation of protein self-assembly *in vivo*.

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IRB Status: None

Disclosures:

JOHN CLARK, PHD: No financial relationships to disclose

COPPER BINDING AND CYTOPROTECTION: ALPHA-CRYSTALLIN AND OTHER SMALL HEAT SHOCK PROTEINS

CHINTALAGIRI MOHAN RAO

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Cu^{2+} has been implicated in several neurodegenerative diseases and in cataract. Cu^{2+} is also known to induce the expression of α A- and α B-crystallin. Prompted by these findings we asked the question "Is there any connection between α -crystallin and copper?" We have demonstrated using fluorescence and isothermal titration calorimetry that α A- and α B-crystallins bind Cu^{2+} with picomolar affinity. Mass spectrometric analysis of peptide fragments bound to a Cu^{2+} -NTA column after in-solution or on-column trypsinolysis show that the Cu^{2+} -binding regions in α B-crystallin are distributed in the N- and C-terminal domains. Recombinant C-terminal domain of α B-crystallin binds 2 Cu^{2+} ions per subunit against 5 Cu^{2+} ions per subunit

of full length α B-crystallin. α -Crystallins inhibit Cu^{2+} -induced oxidation of ascorbate, thereby preventing the generation of reactive oxygen species. We have shown that this property of the α -crystallins confers cytoprotection to IMR-32 human neuroblastoma cells against Cu^{2+} -induced oxidative stress. Other sHsps such as HspB1 (Hsp27), HspB2 and HspB3 are also found in the Lewy bodies and in senile plaques in the brains of patients with Parkinson's or Alzheimer's disease. We have found that these sHsps also bind Cu^{2+} with high affinity, exhibit redox-attenuation and provide cytoprotection. Interestingly, we have observed that sHsps also rescue amyloid beta peptide, A β 1–40, from Cu^{2+} -induced aggregation *in vitro* and extract the Cu^{2+} bound to α -synuclein, thus preventing its Cu^{2+} -induced amyloid fibril formation. Copper also appears to modulate phenotypic outcome of a cataract causing mutation G98R α A-crystallin; Cu^{2+} -binding decreases the chaperone-like activity and increases the aggregation propensity. Properties of Cu^{2+} -binding, redox-attenuation and cytoprotection, that we demonstrated ascribe a novel role to small heat shock proteins in Cu^{2+} -homeostasis and should help in understanding their protective role in neurodegenerative diseases and cataract.

Acknowledgement: JC Bose National Fellowship, Dept. of Science & Technology, New Delhi

IRB Status: None

Disclosures:

CHINTALAGIRI MOHAN RAO, PHD: No financial relationships to disclose

AGE-RELATED CROSSLINKING IN HUMAN LENS PROTEINS: REVELATIONS ON MECHANISMS AND LENS PROTEIN PACKING

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VANDERBILT UNIVERSITY, BIOCHEMISTRY

Lens protein crosslinking has long been considered a key step in the formation of high molecular weight aggregates found in high abundance in aged human lenses. Moreover, these aggregates are believed to be the light scattering centers in age-related nuclear cataracts. Previous studies have focused on the analysis of acid hydrolysates to determine mechanisms of non-disulfide crosslinks. In this study we have used state-of-the-art LC-MS/MS analyses to identify specific non-disulfide protein-protein crosslinks present in aged and cataractous human lenses. Briefly, central nuclear regions of human lenses were homogenized and the proteins were subjected to reduction/alkylation and trypsin digestion. Crosslinked peptides were partially enriched via strong cation exchange chromatography prior to LC-MS/MS analysis using a high resolution orbitrap mass spectrometer. Crosslinked peptides were sequenced and identified using a combination of multiple software tools with manual verification.

Crosslinked peptides were more abundant in cataractous lens nuclei compared to age-matched clear human lenses. Identification of crosslinked peptides revealed several crosslinking mechanisms had taken place including transglutamination and

thioether formation via dehydroalanine. Specific crosslinked peptides identified include: betaB2/betaB2 crystallin, betaA4/betaA4 crystallin, betaA3/betaA4 crystallin, AQP0/AQP0, gammaS/betaB1 crystallin and filensin/betaA3 or betaA4 crystallin. Specific sites of crosslinking provide insight into the molecular structures of crystallins as they pack into the lens fiber cells at high concentrations. Furthermore, specific sites of crosslinking are often observed in regions where crystal structures are indeterminate; thereby providing additional structural information on crystallin aggregates. In conclusion, through the identification of specific crosslinked crystallins we can begin to decipher mechanisms to therapeutically target and to obtain additional structural information currently lacking from crystallography studies.

IRB Status: Verified

Disclosures:

KEVIN SCHEY, PHD: No financial relationships to disclose

O007

ALPHA-A CRYSTALLIN-DERIVED PEPTIDES: POTENTIAL THERAPEUTIC CHAPERONES

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Many of the abnormal properties of the mutant proteins can be controlled by molecular chaperones that are either protein based chaperones or chemical chaperones. The chemical chaperones available have to be used in high concentrations and at these concentrations they can disrupt normal cellular physiology. The chaperone activity of α A-crystallin has been shown to suppress protein aggregation. We have shown that mini-chaperone, (DFVIFLDVKHFSPEDLTVK), a peptide representing the chaperone binding site in α A-crystallin prevents destabilized protein aggregation. The present study was undertaken to improve the mini-chaperone stability, activity and cellular penetration. The peptides—mini-chaperone (DFVIFLDVKHFSPEDLTVK (P1)), C-terminal substituted mini-chaperone (DFVIFLDVKHFSPEDLTVKGRD (P2)), mini-chaperone fused with C-terminal extension of α A-crystallin (DFVIFLDVKHFSPEDLTEEKPTSAPSS (CP-1)) and a cell penetrating form of mini-chaperone (CPP3) were synthesized and supplied by GenScript. Synthetic peptides were tested for chaperone activity using ADH, CS and aLA aggregation assays. The chaperone activity of P2 and CPP3 peptides was 30% greater than the activity of other peptide chaperones tested. We found that the mini-chaperone-stabilized α AG98R displayed chaperone activity comparable to that of wild-type α A-crystallin suggesting rescue of chaperone activity in mutant crystallin. The complexes formed between mini- α A- α AG98R complex and ADH were more stable than the complexes formed between α AG98R and ADH. Western-blotting and mass spectrometry confirmed the binding of mini-chaperone to mutant crystallin. Transduction of all peptide chaperones to Cos7 cells showed no cytotoxic effects. Chaperone peptide labeled with FITC penetrated Cos7 cells very efficiently. The anti-oxidation assay involving H₂O₂ treatment of Cos7 and ARPE-19 cells revealed that all the peptides chaperones tested have cytoprotective property. Based on these observations we conclude that the peptide chaper-

ones have the potential to suppress aggregation of the mutant proteins, rescue the mutant proteins function and control the pathology in protein aggregation diseases.

Support: NIH grant EY023219

IRB Status: None

Disclosures:

KRISHNA SHARMA, PHD: No financial relationships to disclose

LE02 – Can Crystallins Be Used as Therapeutic Agents?

O008

FORMULATION OF CRYSTALLINS AS NANOMEDICINES

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Crystallins, small heat shock chaperone proteins involved in rescuing cells from protein aggregation stress, can be used as therapeutics to treat protein aggregation disorders. The therapeutic application of crystallins is mainly restricted due to their low potency, poor intracellular delivery, and stability issues. Improving the activity, stability, and intracellular delivery of crystallins could be a great potential application in the area of therapeutic development for protein aggregation associated disorders. In this study, we hypothesized that formation of nano/micro assemblies might improve the efficacy, in vivo persistence, and intracellular delivery of crystallins. For this purpose, a variant of alpha crystallin protein (α B-D3) was incubated with different zinc chloride concentration under optimal buffer and pH condition. Addition of zinc chloride induced structural changes in α B-D3 that initiated the formation of stable nano- and micro- supramolecular assemblies. These assemblies showed size-dependent chaperone activity, where nanoassemblies were observed to be 4-fold more effective than the native protein in preventing beta-mercaptoethanol induced aggregation of insulin in in-vitro chaperone assay. Efficacy of insulin (rescued by different sized α B-D3 assemblies during in vitro chaperone assay) was tested in 3T3-L1 adipocytes, wherein insulin rescued by nanoassemblies enhanced glucose uptake in 3T3-L1 adipocytes compared to those rescued by native α B-D3. Further, the most active nanoassemblies (350 nm) showed 1.7-fold improvement in protein stability under urea denaturation, 3-fold higher intracellular delivery, and in vivo persistence for at least two weeks in the rat model. The α B-D3 crystallin nanoassemblies displayed enhanced activity, stability, and delivery compared to the native protein. These unique properties enable α B-D3 crystallin as a promising therapeutic to treat protein aggregation-associated disorders.

IRB Status: None

Disclosures:

ARUN UPADHYAY, PHD: No financial relationships to disclose

THERAPEUTIC POTENTIAL OF SHSP PEPTIDES IN EYE DISEASES

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CASE WESTERN RESERVE UNIVERSITY, OPHTHALMOLOGY AND VISUAL SCIENCES

Small heat shock proteins (sHSP) are molecular chaperones and anti-apoptotic proteins. Sharma and associates first reported peptides within the "α-crystallin domain" of αA-crystallin (HspB4)- and αB-crystallin (HspB5) that showed chaperone properties similar to their parent proteins. We have previously shown that these peptides are anti-apoptotic. We have now identified peptides in two other small heat shock proteins, one in Hsp20 (HspB6) (⁷¹GHFSVLLDVKHFSPEEIAVK⁹¹) and the other in Hsp27 (HspB1) (⁹³DRWRVSLDVNHFAPDELTVK¹¹³). Both peptides inhibited hyperthermia and chemical-induced aggregation of client proteins; in all assays the Hsp20 peptide was better than the Hsp27 peptide. The scrambled peptides of the two proteins showed no such effects. The chaperone activity of the peptides was confirmed by their ability to form complex with client proteins. The use of fluorescein isothiocyanate tagged peptides showed that the peptides in the culture medium are taken up by cells and such uptake was higher in thermally stressed cells. Both peptides inhibited staurosporine-induced apoptosis in HeLa cells by inhibiting cytochrome c release from mitochondria. When the terminal four amino acids (putative domain for cell entry) were scrambled, peptide entry into cells was abrogated and consequently peptides were ineffective in blocking apoptosis. We have previously shown that intraperitoneal (i.p.) injection of the α-crystallin peptides and their lysine-acetylated derivatives completely blocked cataract development in the selenite-cataract rat model and now we extend these findings to show that the i.p. injected peptides of Hsp20 and Hsp27 can also block selenite cataracts. Our preliminary studies show that i.p. injected αB-crystallin peptide is neuroprotective and it inhibits ganglion cell apoptosis in an ischemic retinopathy model. Together, our studies suggest that functional peptides of small heat shock proteins might find use in preventing cataract, and in other eye diseases where apoptosis occurs from ischemia, such as in glaucoma and diabetic retinopathy.

IRB Status: None

Disclosures:

RAM NAGARAJ, PHD: No financial relationships to disclose

MECHANISMS OF AXONAL REGENERATION IN POSTNATAL AND ADULT MONKEY RETINAL GANGLION CELLS

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UNIVERSITY OF MUENSTER, INSTITUTION OF EXPERIMENTAL OPHTHALMOLOGY

Purpose: To study molecular mechanisms involved in regeneration of monkey retinal ganglion cell axons in organotypic retinal cultures in vitro.

Methods: Retinas were obtained from newborn to adult monkeys (*Callithrix jacchus*) immediately after death, freed from surrounding tissue and used to prepare stripes which were cultured in vitro. Growth of axons was monitored using microscopy and time-lapse video cinematography. Immunohistochemistry, Western blotting, qRT-PCR, proteomics and genomics were performed to characterize molecules associated with axonal growth. Then, siRNA experiments were conducted to identify the causal involvement of selected molecules in triggering axonal growth.

Results: We have shown that RGCs exert a growth program with decreasing efficacy throughout life, which becomes fully developed with respect to growth associated molecules and axon specific markers. In particular, receptors to extracellular matrix and specifically for laminin were expressed together with neurofilaments. When the rate of growth was determined with time-lapse videography, it was similar with that reported for embryonic axons, indicating that regenerating axons recapitulate the principal molecular mechanisms of embryonic development. Proteomic profiling of the regenerating retinas in culture showed different patterns from native retinas at matching ages and from retinas without the opportunity to regenerate axons. In particular, proteins involved in Calcium homeostasis such as calmodulin and crystallin beta-b2 were regulated and stress proteins such as heat shock proteins were expressed. The molecular changes associated with axon regeneration in mature RGCs are strikingly similar to those reported during rat ganglion cell axon regeneration or peripheral nerve regeneration (e.g., GAP-43). Genomic profiling using human immobilized cDNA and hybridization with monkey mRNA showed specific regulation of genes belonging to the small nucleoprotein family. Using siRNA for selected molecules, we could partially regain the regenerative status of adult monkey RGCs.

Conclusions: The data show that even after maturation, the molecular mechanisms for axonal growth still exist and can be reactivated to result in stump extension and growth cone formation. Understanding of the molecular mechanisms of axonal regeneration will help to develop therapeutic concepts for optic nerve injuries.

IRB Status: International

Disclosures:

SOLON THANOS, MD, PHD: No financial relationships to disclose

PEPTIDE APTAMERS: POTENTIAL TOOLS TO NEGATIVELY OR POSITIVELY MODULATE HSP27 (HSPB1) FUNCTIONS

ANDRE PATRICK ARRIGO

CLAUDE BERNARD UNIVERSITY LYON

Human HSP27 (HspB1) belongs to the family of small stress proteins, together with alphaB-crystallin (HspB5) and alphaA-crystallin (HspB4). HSP27 is a molecular chaperone which, through dynamic changes in its phosphorylation and oligomerization, allows cells to adapt to changes in their physiology.

A high level of HSP27 expression has been observed in many human pathological conditions. HSP27 can either be beneficial, such as in cellular degeneration diseases, or be malignant in cancers where it promotes tumorigenesis, metastasis and drug resistance. We have shown that HSP27 acts by interacting with specific (and often pathological) client protein partners consequently of changes in its oligomerization and phosphorylation status. These interactions modulate the folding/activity and/or half-life of the clients. Therefore, compounds aimed at either down- or upregulating HSP27 protective activity are actively searched for. In that respect, small interfering RNA or dominant-negative mutants can counteract the antiapoptotic and protective properties of HSP27. Using the peptide aptamer (PA) strategy, we isolated two peptide aptamers (PA11 and PA50) that specifically interact with HSP27 small oligomers and not with the other members of the small heat shock protein family. In mammalian cell cultures, these PAs perturbed the dimerization and oligomerization of HSP27 and acted as negative regulators of its antiapoptotic and cytoprotective activities. Further studies analyzing human tumor xenografts in immunocompromised mice showed that PA11 and 50 strongly reduced tumor development. Of interest, another aptamer (PA23) had the intriguing ability to stimulate the protective activity of HSP27 against different apoptotic drugs. Moreover, this aptamer could abolish the dominant negative effect induced by the R120G mutant of alphaB-crystallin (HSPB5) by disrupting the interaction between HSP27 and mutant HspB5. Hence, structure-based interfering strategies appear as promising approaches that could lead to the discovery of therapeutic drugs modulating small heat shock proteins functions.

IRB Status: None

Disclosures:

ANDRE PATRICK ARRIGO, PHD: No financial relationships to disclose

0012

SMALL HEAT SHOCK PROTEINS ARE EFFECTIVE IN NEUROINFLAMMATORY AND ISCHEMIC DISEASE MODELS

LAWRENCE STEINMAN
STANFORD UNIVERSITY

We have been able to reverse damage with systemic administration of aB crystallin (cryab) in experimental autoimmune encephalomyelitis, anterior optic ischemia, stroke, myocardial infarction and rheumatoid arthritis. Cryab is a protein that forms amyloid aggregates. Amyloid forming molecules are generally considered harmful. In Alzheimer's Disease two amyloid molecules Aβ A4 and tau vie for consideration as the main pathogenic culprit. But molecules obey the laws of chemistry and defy the way we categorize them as humans with our well-known proclivities to bias in our reasoning. We have been exploring the brains of multiple sclerosis patients to identify molecules that are associated with protection from inflammation and degeneration. In 2001 we noted that aB crystallin (cryab) was the most abundant transcript found in MS lesions, but not in healthy brains. Cryab can reverse paralysis and attenuate

inflammation in several models of inflammation including experimental autoimmune encephalomyelitis (EAE), and various models of ischemia. Cryab is an amyloid forming molecule. We have identified a core structure common to many amyloids including amyloid protein Aβ A4, tau, amylin, prion protein, serum amyloid protein P, and cryab. The core hexapeptide structure is highly immune suppressive and can reverse paralysis in EAE when administered systemically. Administration of this amyloid forming hexapeptide quickly lowers inflammatory cytokines in plasma like IL-6 and IL-2. The hexapeptide binds a set of proinflammatory mediators in plasma, including acute phase reactants and complement components. The beneficial properties of amyloid forming hexapeptides provide a potential new therapeutic direction. These experiments indicate that amyloid forming molecules have Janus faces, providing unexpected benefit for neuroinflammatory conditions.

IRB Status: Approved

Disclosures:

LAWRENCE STEINMAN, MD: No financial relationships to disclose

LE03 – Oxidative Stress and the Aging Lens

0013

BIOCHEMICAL CHANGES IN BOVINE LENSES EXPOSED TO HYPERBARIC OXYGEN: IS IT A GOOD MODEL FOR MIMICKING AGE-RELATED NUCLEAR CATARACTS?

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NEW ZEALAND NATIONAL EYE CENTRE, UNIVERSITY OF AUCKLAND, OPTOMETRY AND VISION SCIENCES

Given the difficulty in accessing human cataract lenses, a number of different approaches have been used to create suitable animal models of cataracts. The hyperbaric oxygen (HBO) model has previously been shown to mimic the biochemical changes observed in human age related nuclear (ARN) cataract *in vivo* and *in vitro*. Here we describe the development of this system in bovine lenses since the large size of this lens ensures sufficient material to carry out a number of different assays from just one lens. We exposed young bovine lenses to 100% oxygen (HBO) (or 100% nitrogen (HBN)) at 100 atm for 5 or 15 hours. Lens transparency was assessed using bright and dark field microscopy. Lenses were dissected into the outer cortex, inner cortex and nucleus and GSH/GSSG and MDA levels (a marker of lipid peroxidation) measured. Western blotting of each fraction was used to assess the formation of protein mixed disulfides (PSSG) and loss of actin and AQP0. Immunohistochemistry and confocal microscopy were used to examine lens morphology in the lens nucleus. HBO lenses were hazy with no dense nuclear cataract even after 15 hours. GSH decreased with increased periods of HBO exposure with levels notably reduced in the nucleus relative to the cortex. MDA was increased in HBO compared to HBN lenses in all regions. PSSG formation was observed in the outer and inner cortex of HBO treated lenses and evident in the nucleus only at 15 hours. No signs of actin degradation, loss of AQP0 or

membrane damage were apparent in HBO or HBN lenses which had previously been observed *in vivo*. Overall, our model mimics a number of biochemical changes known to precede the formation of human nuclear cataracts and therefore could be used in the future to test antioxidants effective in delaying the cataract process.

IRB Status: None

Disclosures:

JULIE LIM, PHD: No financial relationships to disclose

O014

IDENTIFICATION OF CRYSTALLIN DISULFIDE BONDS IN CATARACTOUS HUMAN LENSES

LARRY DAVID, Phillip Wilmarth, Eileen Yue

OREGON HEALTH AND SCIENCE UNIVERSITY, BIOCHEMISTRY AND MOLECULAR BIOLOGY

Increased disulfide bonds between crystallins are a hallmark of age-related human cataract. Identifying the specific cysteines that are paired in disulfides is important, because it provides structural information about the light scattering water-insoluble aggregates found in age-related cataracts. These studies introduce an improved method to identify disulfide cross-links using high-resolution mass spectrometry and software designed to interpret data from crosslinking experiments. Water-insoluble protein from a 93 year-old cataractous human lens was alkylated with idoacetic acid either with or without prior reduction, digested with trypsin and peptides separated by a 200 min 2-30% acetonitrile gradient on a 75 μ m x 50 cm EasySpray C18 UPLC column. Peptides were analyzed using an Orbitrap Fusion mass spectrometer to acquire survey scans at 120,000 resolution, and data-dependent MS2 scans using quadruple isolation, exclusion of charge states $< +3$, HCD fragmentation, and 60,000 resolution. Disulfide cross-links were then identified using StavroX software with precursor and fragment ion tolerances of 2 and 5 ppm, respectively. Disulfide linked peptides required a StavroX score of 4 or greater for identification. Fifteen different disulfide cross-links were identified by StavroX with scores greater than 4: $\beta B1_{C79}-\beta B1_{C79}$; $\beta A3_{C170}-\beta A3_{C185}$; $\beta B1_{C79}-\beta A4_{C32}$; $\gamma C_{C22}-\gamma C_{C32}$; $\gamma D_{C18}-\gamma D_{C32}$; $\gamma D_{C32}-\gamma D_{C41}$; $\gamma D_{C18}-\gamma D_{C78}$; $\beta B1_{C79}-\gamma S_{C36}$; $\beta B1_{C79}-\gamma S_{C82}$; $\beta A4_{C32}-\gamma S_{C82}$; $\gamma C_{C22}-\gamma S_{C82}$; $\beta B1_{C79}-\gamma S_{C129}$; $\beta A4_{C32}-\gamma S_{C129}$; $\gamma C_{C22}-\gamma S_{C129}$; $\gamma S_{C36}-\gamma S_{C129}$ while no disulfide peptides scored above 1 in the reduced sample. Seven of these 15 disulfide cross-links were between γS and other crystallins, suggesting that γS plays a key role in forming disulfide linked aggregates in the lens. Three of these γS disulfide linkages were also with the single C79 of $\beta B1$ crystallin. This suggests that $\beta B1$ and γS form an interface in the light scattering aggregates of cataractous lens.

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IRB Status: Verified

Disclosures:

LARRY DAVID, PHD: No financial relationships to disclose

O015

PARKIN-DIRECTED MITOPHAGY GOVERNS LENS EPITHELIAL CELL MITOCHONDRIAL DEGRADATION UNDER OXIDATIVE STRESS CONDITIONS

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FLORIDA ATLANTIC UNIVERSITY, COLLEGE OF MEDICINE

Mitochondrial function is essential for the homeostasis and transparency of the eye lens and it has recently been shown that mitophagy is used by the lens to degrade mitochondria. The E3 ubiquitin-ligase Parkin is expressed by the lens epithelium and is known to target damaged mitochondria for degradation in other systems. We hypothesized that Parkin is required to maintain functional lens epithelial cell mitochondrial populations and viability under oxidative stress conditions.

To test this hypothesis, dual-label fluorescent confocal microscopy and western blot analysis were used to identify the translocation of Parkin and its ability to initiate the degradation of oxidized mitochondria in human SRA 01/04 and primary chick lens epithelial cells.

The results demonstrated that Parkin transcript was induced by H_2O_2 -treatment in both SRA 01/04 and cultured primary chick lens epithelial cells. Treatment of SRA 01/04 or primary chick epithelial cells with the mitochondrial membrane depolarizing agent CCCP or H_2O_2 resulted in translocation of Parkin to the mitochondrial membrane. Overexpression of wild-type Parkin in lens epithelial cells exposed to CCCP or H_2O_2 -treatment resulted in complete elimination of damaged mitochondria. By contrast, overexpression of a mutant form of Parkin that lacks ubiquitin ligase activity in lens epithelial cells exposed to CCCP or H_2O_2 -treatment resulted in Parkin translocation without accompanied mitochondrial degradation. Failure to degrade damaged mitochondria in lens epithelial cells resulted in increased reactive oxygen species (ROS) production and loss of lens epithelial cell viability.

The results demonstrate that Parkin governs the degradation of damaged lens epithelial cell mitochondria and that Parkin function is critical for the maintenance of functional lens epithelial cell mitochondrial populations and viability. Collectively, these data implicate Parkin in lens homeostasis and prevention of cataract formation.

IRB Status: None

Disclosures:

LISA BRENNAN, PHD: No financial relationships to disclose

O016

THE THIOL OXIDATION REPAIR ENZYME GLUTAREDOXIN CONTROLS SENILE CATARACT FORMATION

MARJORIE LOU

UNIVERSITY OF NEBRASKA-LINCOLN SCHOOL OF VETERINARY MEDICINE AND BIOMEDICAL SCIENCES

Glutaredoxin (Grx) belongs to the oxidoreductase family and has isoforms of cytosolic Grx1 and mitochondrial Grx2. It has dethiolase activity that reduces the disulfide bridge of protein-thiol mixed disulfide (or PSSG) formed during oxidative stress, and restores proteins/enzymes functions/activity. Thus, Grx is an important protein thiol damage repair system in the lens in which the homeostasis of SH/-SS- is regulated to protect against oxidative stress. The cytosolic Grx1 or thioltransferase (TTase) in the lens also displays dehydroascorbate reductase and peroxidase activities that maintain the reduce status of ascorbate and lipids, respectively. TTase has a physiological function in regulating cell proliferation via its interaction with phosphatases, and in glycolytic ATP generation via regulating glyceraldehyde 3-phosphate dehydrogenase activity. Grx1 gene knockout (KO) in mice (Grx1 KO) shows a phenotype of faster (3-4 m) and more severe cataract formation during aging compared with the age-matched wild type controls. These Grx1 KO lenses have large protein aggregates with high levels of PSSG accumulation in structure proteins, including α A crystallin and β -actin. Grx1 KO mice also display high sensitivity to UVB irradiation stress. The mitochondrial Grx2 shares the same catalytic properties as TTase. It is a crucial enzyme in protecting cells against oxidation-induced mitochondrial damages, which include compromised ATP production from inactivated Complex I, cytochrome c leakout and cell apoptosis. Grx2 gene KO mice also display faster lens opacity during aging, parallel to that of Grx1 KO mice. In conclusion, Grx is a key anti-oxidation enzyme in the lens. Its ability to repair lens proteins/enzymes from oxidative damage is essential to lens transparency.

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IRB Status: Verified

Disclosures:

MARJORIE LOU, PHD: No financial relationships to disclose

0017

MULTIFUNCTIONAL ANTIOXIDANTS FOR THE TREATMENT OF AGE-RELATED OXIDATIVE CHANGES IN THE LENS AND RETINA

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Orally active multifunctional antioxidants (MFAOs) possessing distinct free radical scavenging activity and independent metal attenuating activity have been synthesized and evaluated in ocular cell lines and animal models of cataract and retinal degeneration. In rats these have been shown to protect the lens against ROS generated by ER stress, gamma, and UV irradiation and in the retina, protect the photoreceptor cells against light damage. In cell lines such as the human retinal pigmented epithelial (RPE) cells, these compounds have been shown to protect the cells against mitochondrial damage induced by manganese chloride as evidenced by rhodamine staining and neurotoxicity induced by A β :Zn complex as evidenced by Zinquin staining. Interestingly, these compounds can remove Zn from the tightly bound A β :Zn complex but allow free Zn levels to be maintained in the cellular cytoplasm. These studies

suggest that MFAOs represent a new class of drugs for the treatment of age related ocular diseases such as cataract and macular degeneration.

IRB Status: Approved

Disclosures:

PETER KADOR, PHD: Patents/Royalties relationship with University of Nebraska Medical Center; Equity Owner relationship with DrugsforEyes LLC

LE04 – Communication in the Eye: Channels and Transporters

0018

A CX46 FRAME-SHIFT MUTANT, CX46FS380, CAUSES PROGRESSIVE CATARACTS IN THE MOUSE LENS

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UNIVERSITY OF CHICAGO

A connexin46 (Cx46) mutant, Cx46fs380, has been linked to inherited human cataracts. This mutant has a frame-shift resulting in a protein that has an aberrant sequence in its carboxyl terminus and is 31 amino acids longer than wild type Cx46. To test the consequences of expression of Cx46fs380 in the lens, we generated knock-in Cx46fs380 mice. Lenses were examined by darkfield and immunofluorescence microscopy. Although lenses were transparent at 1 month of age, anterior cataracts were observed at 2 months in homozygotes and in some, but not all, heterozygotes at 4 months. At later times, all mutant mice had cataracts that became progressively worse with age. Levels of several lens proteins including Cx46 and Cx50 (the co-expressed connexin) were determined by immunoblotting. Cx46 was dramatically decreased in both heterozygous and homozygous Cx46fs380 lenses at 1 month of age. Cx50 levels were reduced in young mutant mice and became even lower with age. While in old Cx46fs380 homozygotes aquaporin0 levels were unaffected, N-cadherin levels were decreased. Alpha-, beta-, and gamma-crystallins were water soluble in young animals. While the beta- and gamma-crystallins were partially water-insoluble in older wild type mice, the insoluble fraction was greater in heterozygous and homozygous mutant mice. These results demonstrate that expression of one Cx46fs380 allele is sufficient to induce cataracts. Moreover, the severe decrease in Cx46 precedes the appearance of cataracts, suggesting that these animals will be a good model to elucidate the time course of alterations in lens components associated with a Cx46 mutant.

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MECHANISMS REGULATING INTRACELLULAR HYDROSTATIC PRESSURE AT THE SURFACE OF THE MOUSE LENS

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SUNY AT STONY BROOK¹; UNIVERSITY OF ARIZONA AT TUCSON, PHYSIOLOGY²

The intracellular leg of fluid circulation in the lens is driven by a hydrostatic pressure gradient from around 350 mmHg in central fiber cells to 0 mmHg in surface cells. Recent studies have shown surface cell pressure (p_i) increases in lenses lacking PTEN, the phosphatase for PI3K. This causes eventual rupture of the lenses, indicating zero p_i is important and that feedback control normally regulates p_i . This study examines the feedback control system responsible for maintaining zero pressure in surface cells. Hydrostatic pressures were measured with an intracellular microelectrode and a manometer based system. When the Na/K ATPase was partially inhibited, p_i became positive but within 2 hours returned to zero. Inhibition of TRP4 channels did not affect p_i in control conditions, but when p_i became positive, it did not return to zero when TRP4 channels were inhibited. Activation of TRP4 channels caused p_i to go negative but within 2 hours it returned to zero. Inhibition of the PI3K-Akt pathway did not affect p_i in control conditions, but when p_i was negative, it did not return to zero when Akt was inhibited. In summary, fluid leaves lens surface cells through a combination of hydrostatic and osmotic pressures ($p_i - RT\Delta c_i$). Normally p_i is controlled through the Na/K ATPase, which adjusts Δc_i to maintain fluid exit. TRP4 channels are inactive when $p_i = 0$, however when p_i becomes positive, they sense the positive pressure and activate. This initiates a signal transduction cascade that eventually stimulates the Na/K ATPase, thus reducing Δc_i and restoring p_i to zero. If p_i becomes negative, a different (yet to be identified) sensor is activated. This sensor initiates a signal transduction cascade that activates PI3K and Akt to inhibit Na/K ATPase activity, thus increasing Δc_i and restoring p_i to zero.

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THE REGULATION OF CATION CHLORIDE COTRANSPORTERS IN THE LENS

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Lens transparency is dependent on strict cell volume regulation to maintain its highly ordered tissue architecture. Previously, our laboratory has determined that members of the Cation Chloride Cotransporter (CCC) family, that includes the K-Cl-cotransporter (KCC), the Na-K-Cl-cotransporter (NKCC) and the Na-Cl-cotransporter (NCC), play key roles in lens volume regulation¹. In other tissues, steady state cell volume is maintained by balancing ion efflux and influx mediated by KCCs and NKCCs, respectively. This reciprocal regulation of CCC's is controlled by the integrated activity of a group of kinases and phosphatases that in response to changes in cell volume modulate the transport activity of the transporters by altering their phosphorylation status². In response to cell shrinkage a signaling pathway, comprising the kinases With no Lysine Kinase (Wnk1, 3, 4), Ste-20 like Proline/Alanine rich Kinase (SPAK) and Oxidative Stress Response Kinase 1 (OSR1), is activated that phosphorylates the transporters thereby increasing NKCC mediated ion influx and decreasing KCC mediated efflux to effect an increase in cell volume. Conversely in response to cell swelling the kinase activity is reduced and the phosphatases PP1 and PP2A dephosphorylate the transporters to produce a decrease in NKCC mediated ion influx and an increase in ion efflux via KCC that reduces cell volume. We now report that these same kinases are expressed in the lens and that altering lens volume produces a change in the phosphorylation status of CCC's in the lens. Since fibre cell swelling initiates diabetic cataract¹, our identification of the signaling pathway that controls the transporters that set lens cell volume has the potential to offer new insights into the pathogenesis of diabetic lens cataract.

This work was supported by the HRC and the AMRF

1. Donaldson et al., *Exp Eye Res.* 2009;88:144-150

2. Kahle et al., *Annual Review of Physiology.* 2008;70:329-55.

IRB Status: None

Disclosures:

PAUL DONALDSON, PHD: No financial relationships to disclose

AQUAPORIN ZERO REGULATION AND ROLES IN LENS DEVELOPMENT AND HOMEOSTASIS

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Background and aims: Aquaporin zero (AQP0) is essential for the proper development and maintenance of clarity of the optical lens. Zebrafish and other teleost fish have two divergent copies of AQP0 (a and b) due to a genome duplication event that occurred over 300 million years ago. Aqp0a is a regulated water channel, but Aqp0b is not. Our aim is to understand the roles of AQP0 and the mechanisms of the regulation of its water permeability in lens development and homeostasis.

Methods: We express aquaporins in *Xenopus* oocytes or other appropriate expression systems and measure water permeability and adhesion. To assess the biological roles of mutant and wild type aquaporins, we use the zebrafish (*Danio rerio*) model. We knock down endogenous proteins using morpholino oligonucleotides or genetic constructs and express rescue proteins using genetic constructs driven by a lens-specific gamma crystalline promoter.

Results: We have examined the effects of eliminating water permeability itself or its regulation by calcium/calmodulin (CaM) on the ability of exogenous Aqp0a to rescue lens defects (cataracts) in Aqp0a or Aqp0b deficient zebrafish larvae. We find that while regulation-deficient constructs or constructs entirely lacking water permeability do not rescue Aqp0a-deficient larvae, they do rescue Aqp0b deficiency, further confirming that Aqp0b has essential functions in the lens other than as a water channel.

Conclusions: Our results confirm that AQP0 has multiple separable functions (all present in MIPfun and presumably also in mammalian AQP0) only one of which is water permeability. Regulation of water permeability is essential, at least for the development of the lens, but it is not clear what roles water permeability or its regulation play in the adult lens. We will present current efforts to refine our understanding of the mechanism of CaM regulation of AQP0 water permeability.

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O022

RELATIONSHIP BETWEEN STRUCTURE OF THE MONOCARBOXYLATE TRANSPORTER MCT12 AND ITS FUNCTION IN CATARACT FORMATION AND CREATINE TRANSPORT

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Recently we have shown that the cataract-associated monocarboxylate transporter MCT12, encoded by the gene *SLC16A12*, is a creatine transporter. Screening of the DNA from patients with either congenital or age-related cataract resulted in the identification of 12 mutations which map to the coding region of *SLC16A12*, most of which are amino acid substitutions. In order to assess their contribution to creatine transport, we introduced the mutations in expression constructs and tested their ability to transport creatine in the heterologous *Xenopus laevis* oocyte system. Further, we modeled the likely structure of the transporter and compared it to the predicted effects of amino acid substitutions found in patients. We were able to map creatine binding sites by making use of different algorithms. Furthermore, the amino acid substitutions yielded alter-

ations of the overall structure and showed effects on creatine transport. Our studies evaluate first assessments of a structure-function relationship and how different DNA sequence variants affect function of this transporter.

IRB Status: International

Disclosures:

BARBARA KLOECKENER-GRUISSEM, PHD: No financial relationships to disclose

O023

METABOLIC COUPLING BETWEEN THE RETINA PIGMENT EPITHELIUM AND THE NEURAL RETINA: THE RPE PRODUCES BETA-HYDROXYBUTYRATE TO SUPPORT RETINAL METABOLISM

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THOMAS JEFFERSON UNIVERSITY¹; THOMAS JEFFERSON UNIVERSITY, PATHOLOGY, ANATOMY, CELL BIOLOGY²

In the outer retina, the RPE and photoreceptor cells are physically and metabolically coupled and this symbiotic relationship supports normal visual function. The RPE phagocytizes shed photoreceptor OS (POS) and digest them in phagolysosomes generating fatty acids that the RPE can utilize as an energy substrate. From microarray analysis of various mouse tissues, we found that RPE cells express particularly high levels of the mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase 2 (Hmgcs2) compared to all other tissues (with the exception of the liver and colon), leading to the hypothesis that RPE cells can produce β -HB from metabolism of fatty acids. Using primary human fetal RPE (hfrPE) cells cultured on transwell filters with separate apical and basal chambers, we demonstrate that hfrPE cells can metabolize palmitate, a saturated fatty acid that constitutes $\approx 15\%$ of all lipids in rat POS, to produce β -HB. Importantly, we found that hfrPE cells preferentially release β -HB into the apical chamber. In freshly isolated mouse RPE cells, incubation with palmitate also resulted in significantly higher β -HB release into the supernatant compared to the no-substrate control. We show that in hfrPE cells, β -HB transport across the apical membrane is mediated primarily by monocarboxylate transporter isoform 1 (MCT1). Using GC-MS analysis of ¹³C-labeled metabolites, we showed that retinal cells can take up and metabolize ¹³C-labeled β -HB into various TCA cycle metabolites (e.g., citrate and α -ketoglutarate) and amino acids (e.g., alanine and glutamate). Furthermore, we demonstrate this process is inhibited by the specific MCT1 inhibitor (AR-C155858), suggesting that a fraction of β -HB taken up by the retina is metabolized in photoreceptor cells. Taken together, our data support a novel mechanism of RPE-retina metabolic coupling in which RPE cells metabolize fatty acids to produce β -HB that are transported to the retina to be used as a metabolic substrate.

IRB Status: None

Disclosures:

NANCY PHILP, PHD: No financial relationships to disclose

HUMAN OCULAR TRANSPORTERS: TRANSPORTER-MEDIATED INTRAOCULAR DRUG DELIVERY

SUNIL VOOTURI

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Intraocular drug delivery is a major challenge because of the unique protective barriers that are present in the eye. One approach to enhance the intraocular drug delivery is to use the body's own solute transporters and design prodrugs that are structurally similar to the transporter substrates. Transporter-mediated drug delivery has been extensively characterized for oral and brain delivery. On the other hand, there is a dearth of knowledge in the literature for the characterization and utilization of drug transporters in ocular drug delivery. Researchers have shown the presence of solute transporters in ocular barriers using isolated cell cultures and preclinical animal models. However, there are limited reports showing the characterization of transporters in human ocular tissues. Our lab has characterized few important solute transporters in the human ocular barriers that can be potentially used for enhancing drug delivery. Further, our laboratory has designed and developed novel ocular prodrugs to confirm the feasibility of transporter-mediated approach to enhance the intraocular drug delivery.

IRB Status: Approved

Disclosures:

SUNIL VOOTURI, PHD: No financial relationships to disclose

LE05 – Cataract Genetics and Genomics

CATARACT GENE DISCOVERY VIA NEXT-GENERATION SEQUENCING

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Inherited forms of cataract are a clinically important and genetically heterogeneous cause of visual impairment. Typically they present at an early age, with or without other ocular/systemic signs, and lack clear phenotype-genotype correlation rendering both clinical classification and molecular diagnosis challenging. Here we have utilized targeted next-generation sequencing (NGS) to discover gene mutations underlying autosomal dominant cataract segregating in nuclear (trio/quartet) and multi-generation families. Following ethical approval and informed consent, genomic DNA fragment-libraries were prepared from donor blood samples, and subjected to whole-exome or custom-target hybridization with RNA-capture probes followed by paired-end NGS. After alignment of NGS reads to the reference human genome, and stringent bioinformatics filtering, novel variants were validated by Sanger sequencing and further analyzed in silico to predict likely effects on protein function. Two novel missense mutations, predicted to 'damage'

connexin-50 function, were found in GJA8, and a recurrent missense mutation, known to reduce the solubility of gamma-D crystallin, was found in CRYGD. In addition, we detected the first mutation in a novel gene for cataract that encodes a non-selective cation channel believed to function in cellular calcium signaling/homeostasis. This novel missense transition resided within an alternatively spliced exon that was present in multiple transcript variants of the gene. Expression in vitro of a GFP-fusion product predicted that the resulting amino-acid substitution (Met) introduced an alternative translation start-site in several protein isoforms; raising the likelihood of deleterious gain-of-function effects on protein function. Overall, targeted NGS provides an effective means to profile mutations in over 50 genes that are known to underlie inherited forms of cataract. Discovery of novel cataract genes is also facilitated by NGS. However, considerable supporting evidence (e.g. linkage analysis, additional mutations, functional expression in vitro, and/or an animal model) is required to verify disease causation.

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Disclosures:

ALAN SHIELS, PHD: No financial relationships to disclose

WHOLE-EXOME SEQUENCING OF PEDIATRIC CATARACT: LESSONS LEARNED, NOVEL CANDIDATES, AND FUTURE DIRECTIONS

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Whole exome sequencing analysis of 32 probands and 21 family members from pedigrees affected with pediatric cataracts will be presented; 24 out of 32 families demonstrated a dominant inheritance pattern. Pathogenic and likely pathogenic mutations were identified in 12 families and comprised missense (7), nonsense (3) and frameshift (2) mutations in *CRYAA*, *CRYBA2*, *CRYBB1*, *CRYBB3*, *CRYGC* (2), *CRYGD*, *EPHA2*, *EYA1*, *GJA8* (2) and *MIP*. Mutations in additional genes including *BCOR*, *CRYBB2* and *COL4A1* are currently under investigation including testing family members for co-segregation. Several potential novel cataract factors have been identified including *PAH*, *LENG8*, *SLC7A8* and others. These factors are being studied in additional cataract families and animal models to further investigate their potential involvement in lens development and cataract. Our study demonstrates that 1) less than half of pediatric cataracts can be explained by mutations in currently known genes; 2) congenital cataracts are a highly heterogeneous disorder (each family in our study had a unique mutation and no one gene explained more than two cases); 3) missense alleles represent the largest mutation class in congenital cataracts, similar to other conditions; 4) multiple cataract genes demonstrate both dominant and recessive inheritance patterns; 5) some mutations are associated with incomplete penetrance and/or later onset. Thus, interpretation of whole exome data and identification of causative cataract mutations encounters a number of challenges. Discovery of additional cataract factors

and functional studies of known and novel cataract genes/alleles is of high priority to provide better understanding of normal lens development and cataract formation.

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Disclosures:

ELENA SEMINA, PHD: No financial relationships to disclose

0027

CHARACTERIZATION OF A DNA-REPAIR GENE (*ERCC2*) IN A RECESSIVE CATARACT IN THE MOUSE – UNEXPECTED FINDING BY EXOME SEQUENCING

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Cataracts are the major eye disorder and have been associated mainly with mutations in lens-specific genes, but they are also frequently associated with complex syndromes. In a large-scale high-throughput ENU mutagenesis screen we analyzed the offspring of paternally treated C3HeB/FeJ mice for obvious dysmorphologies.

We identified a mutant suffering from rough coat and small eyes only in homozygotes; homozygous females turned out to be sterile. The mutation was mapped to chromosome 7 between the markers *116J6.1* and *D7Mit294*; 4 other markers within this interval did not show any recombination among 160 F2-mutants. The critical interval (8.6 Mb) contains 3 candidate genes (*Apoe*, *Six5*, *Opa3*); none of them showed a mutation. Using exome sequencing, we identified unexpectedly a c.2209T>C mutation in the *Xpd/Ercc2* gene leading to a Ser737Pro exchange.

During embryonic development, the mutant eyes did not show major changes. Postnatal histological analyses revealed small vacuoles at the anterior pole of the lens; later, cortical cataracts developed as observed. Since *Xpd/Ercc2* is involved in DNA repair, we checked also for the presence of the repair-associated histone gamma-H2AX in the lens. During the time, when primary lens fiber cell nuclei are degraded, gamma-H2AX was strongly expressed in the anterior part of the lens fiber cells; however, its expression was diminished in heterozygotes and almost absent in the homozygous mutants indicating that *Xpd/Ercc2* is an important factor for terminal differentiation of lens fiber cells.

These findings demonstrate the importance of *Xpd/Ercc2* for lens fiber cell differentiation. Since epidemiological data are controversial about the association of SNPs in the *XPD/ERCC2* gene with cataracts, our study let us conclude that such an association might be expected. Moreover, we hypothesize that variations in the human *XPD/ERCC2* gene might increase the susceptibility for cataracts in heterozygotes under particular environmental conditions.

IRB Status: International

Disclosures:

JOCHEN GRAW, PROF DR: No financial relationships to disclose

0028

POSITIONAL CLONING OF THE GENE FOR HEREDITARY CATARACT IN THE NAKANO MOUSE REVEALS INVOLVEMENT OF THE HEME BIOSYNTHESIS PATHWAY IN LENS PHYSIOLOGY

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Positional cloning is a powerful method of gene identification in which a gene for a specific phenotype is identified only by its approximate chromosomal location. The Nakano cataract (NCT) is one of the oldest mutant mouse models for hereditary cataract. Despite what is known about cataract-associated morphological and biochemical changes, the culprit genetic defect underlying cataractogenesis in Nakano mice was not determined. Nakano mice develop cataracts as an autosomal recessive trait governed by a single locus, referred to as *nct*. Then, we attempted positional cloning of the *nct* gene. Initially, we performed chromosomal mapping of the *nct* locus by breeding BALB/*c-nct/nct* × (BALB/*c-nct/nct* × MSM/Ms) backcross mouse progeny, and demonstrated that the locus is located in a region between 2 microsatellite marker loci, *D16Mit5* and *D16Mit185*, on chromosome 16. BLAT search of the marker sequences on the mouse genome revealed that 38 genes are located in the critical region. We then performed RT-PCR analysis to examine mRNA transcripts for 9 of the 38 *nct* candidate genes in the BALB/*c-nct/nct* lens, and found a causative mutation in the coproporphyrin oxidase gene (*Cpox*), encoding the enzyme responsible for catalyzing oxidative decarboxylation of coproporphyrinogen III in the heme biosynthetic pathway. BALB/*c-nct/nct* mice have a G to T nucleotide substitution in the *Cpox* gene, which results in a p.R380L amino acid substitution in the CPOX protein. The CPOX isoform with the p.R380L substitution retained only 15% of the activity of the wild type isoform. BALB/*c-nct/nct* mice had excessive accumulation of coproporphyrin III in the lens. The NCT phenotype was normalized by the introduction of a wild type *Cpox* transgene. The mechanisms by which impairment of CPOX leads to lens opacity in the NCT are elusive. However, our data illuminate a hitherto unanticipated involvement of the heme biosynthesis pathway in lens physiology.

IRB Status: None

Disclosures:

MASAYUKI MORI, PHD: No financial relationships to disclose

0029

MOLECULAR ANALYSIS OF CONGENITAL CATARACT CAUSING MUTATIONS IN THE *EPHA2* GENE

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Congenital cataract is one of the leading causes of childhood blindness. Mutations in the EPHA2 gene lead to inherited congenital cataract in families from different ethnicities including in Australian families reported by our group. The EPHA2 gene encodes a transmembrane tyrosine kinase receptor with an extracellular, a transmembrane and an intracellular region. The latter comprises of juxtamembrane, tyrosine kinase, sterile- α -motif (SAM) and PDZ (<u>P</u><u>SD-95, <u>D</u><u>LG, <u>Z</u><u>O-1) domains. EPHA2 is highly expressed in epithelial cells and localizes to the cell membrane. It interacts with cell-junction proteins and plays an important role in development and maintenance of epithelia. Congenital cataract causing mutations in EPHA2 can affect the formation and integrity of cellular contacts in the lens. We used an in vitro epithelial cell culture system to examine effect of 5 causative mutations affecting amino acid residues in the juxtamembrane domain, SAM domain or the segment between SAM and PDZ domains of EPHA2. Mutations were introduced into wild-type myc-tagged EPHA2 cloned in pQCXIP vector by PCR-based mutagenesis. The localization of ectopically expressed myc-tagged EPHA2 was analyzed in Madin-Darby Canine Kidney (MDCK) and human epithelial colorectal adenocarcinoma (Caco-2) epithelial cells by immunofluorescence labelling. A previous study in fibroblast cells showed that congenital cataract causing mutations in EPHA2 lead to protein aggregation upon receptor stimulation. However, we found that 2 out of the 3 mutations located within the SAM domain led to perinuclear localization of the protein in both the epithelial cell types. All the other EPHA2 mutant proteins, like the wild-type, localized to the cell membrane. The mislocalisation of mutant protein with altered residues in the SAM domain suggests that these mutations could affect protein function at cellular junctions. Further studies are required to establish these functional effects.

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ALPANA DAVE, MBIOTECH: No financial relationships to disclose

O030

A PHARMACOLOGICAL CHAPERONE FOR A CHAPERONE PROTEIN: REVERSAL OF CATARACTS CAUSED BY A MUTATION IN α B-CRYSTALLIN

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UNIVERSITY OF CALIFORNIA, SAN FRANCISCO¹; WASHINGTON UNIVERSITY, OPHTHALMOLOGY AND VISUAL SCIENCES²

The molecular chaperone α B-crystallin is highly expressed in the eye lens, where it helps to maintain the transparency of lens proteins over an individual's lifetime. The mutation R120G promotes the aggregation of α B-crystallin into amyloid fibrils and causes a hereditary form of cataract. The goal of this work is to develop a small molecule inhibitor of R120G amyloid formation as a potential nonsurgical treatment for cataract. We

utilized differential scanning fluorimetry to identify a class of small molecules that partially rescue the wild-type stability of R120G α B-crystallin. Direct binding to R120G α B-crystallin was confirmed using biolayer interferometry and HSQC NMR. The effect of the lead compound on the aggregation and disaggregation of R120G was characterized using transmission electron microscopy, and its *in vivo* efficacy was assessed in R120G knock-in mice. Electron microscopy revealed that the lead compound c29 prevents R120G amyloid fibril formation and disrupts preformed fibrils, while an inactive analog does not. When administered to R120G knock-in mice via eyedrop three times per week for two weeks, c29 improved lens transparency in 83% of heterozygous R120G mice and 86% of homozygous mice. The solubility of α , β , and γ -crystallins increased by 63% relative to the vehicle-only control, and total protein solubility improved by $16 \pm 5\%$. Finally, in lens material from human patients with grade 3 to 4 cataracts, compound 29, but not the vehicle treatment, significantly improved the ratio of soluble protein from 0.122 to 0.153 (a 26% increase). These results suggest that c29 is a pharmacological chaperone for α B-crystallin and may be a viable therapeutic intervention in congenital and age-related cataracts. Future work will continue to evaluate the efficacy, pharmacokinetics, toxicity, and mechanism of action of c29.

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LEAH MAKLEY, BS: No financial relationships to disclose

LE06 – Death and Differentiation: Novel Pathways in the Lens

O031

SUPPRESSION OF JNK-MTOR SIGNALING PATHWAY INDUCES PREMATURE FORMATION OF THE ORGANELLE FREE ZONE BY AUTOPHAGY DURING LENS FIBER CELL DIFFERENTIATION

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THOMAS JEFFERSON UNIVERSITY

Autophagic pathways are essential to many developmental processes, yet the initiation signals that regulate autophagy in the context of differentiation are not well understood. Recent studies revealing the presence of autophagic processes in the lens suggested that the precisely regulated spatiotemporal formation of the organelle free zone (OFZ) during lens development could be a good model for investigation of autophagy-linked signaling in the context of differentiation. We first showed that in the chick embryo lens organelles are removed from the central fiber zone in a pathway that involves disposal in autophagic vesicles. Then, using the developing chick embryo lens as our model, we discovered a novel role for the MAP kinase JNK in regulating the timing of removal of lens

organelles for formation of the OFZ. In this pathway, the JNK "on signal" is required for both activation of MTOR and phosphorylation of RAPTOR, both elements of MTORC1 complex that is central to preventing autophagy induction. We found that inactivation of JNK and the MTORC1 complex is linked to formation of the lens OFZ in vivo. Using an organ culture system we showed that inactivation of JNK in lenses that had not yet initiated formation of the OFZ leads to inactivation of the MTORC1 complex through a pathway involving both inactivation of MTOR and loss of phosphorylation of RAPTOR. Inactivation of this signaling pathway, achieved either by blocking either JNK or MTOR signaling, was found to induce premature formation of the lens OFZ through the activation of an endogenous autophagic pathway. Our results reveal that JNK is a positive regulator of MTORC1 signaling and its developmentally regulated inactivation provides an inducing signal for the coordinated autophagic removal of organelles required for lens development and function.

IRB Status: None

Disclosures:

SUE MENKO, PHD: No financial relationships to disclose

0032

INTEGRIN α V β 5-MEDIATED PHAGOCYTOSIS BY LENS EPITHELIAL CELLS INCREASES CELL SURVIVAL UNDER APOPTOTIC CONDITIONS

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The lens epithelium is required to maintain the transparent function of the entire lens but is routinely exposed to apoptosis-inducing UV-light. Since the lens has no known source of professional phagocytes to remove damaging apoptotic cells and toxic debris, we hypothesized that lens epithelial cells must have evolved at least one mechanism to eliminate apoptotic cellular debris and thereby preserve the viability of the overall epithelial cell population. Here, we used fluorescent confocal microscopy, transmission electron microscopy and dual-fluorescent cell labeling techniques to show that lens epithelial cells can phagocytize apoptotic cell debris and other substrates and we used a function-blocking antibody (P1F6) and a specific Rac1 (Ras-related C3 botulinum toxin substrate 1) inhibitor (NSC23766) to show that lens epithelial cell phagocytosis requires the functions of integrin α V β 5 receptor and Rac1. Importantly, we demonstrate that reducing the actions of integrin α V β 5 or Rac1 results in significantly decreased lens epithelial cell survival under apoptotic conditions. These results demonstrate that integrin α V β 5-mediated phagocytosis is a novel mechanism for preserving lens epithelial cell populations under apoptotic conditions and suggest that phagocytosis by lens epithelial cells is a mechanism for lens cell survival in the face of UV-light induced apoptosis and potentially other cataract-associated insults.

IRB Status: None

Disclosures:

MARC KANTOROW, PHD: No financial relationships to disclose

0033

AUTOPHAGY AND THE UNFOLDED PROTEIN RESPONSE IN MOUSE MODELS OF HEREDITARY CATARACTS

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α A- and α B-crystallin are lens chaperones that play an essential role in the transparency and refractive properties of the lens. Mutations in α A- and α B-crystallin are associated with the development of hereditary human cataracts. The R49C mutation of α A-crystallin was identified in four generations of a Caucasian family with hereditary cataracts. The α A-R49C protein forms larger than normal oligomers in the lens, has decreased solubility, and causes cell death in the lens epithelium. Aberrant α A-R49C oligomerization suggests that altered protein folding plays a role in cataract pathogenesis. We investigated the unfolded protein response (UPR) activation in an in vivo mouse model of α A-R49C and found that the expression of the endoplasmic reticulum chaperone, BiP was 5-fold higher in homozygous α A-R49C lenses than in wild type lenses. Analysis of proteins typically expressed during the UPR revealed that ATF-4 and CHOP levels were also higher in the lenses of mutant mice, while the opposite was true of ATF-6 and XBP-1. These findings show that the PERK pathway components of UPR are an important mediator of cell death observed in α A-R49C lenses. The R120G mutation in the α B-crystallin gene is also associated with hereditary human cataract. The α B-R120G protein forms larger than normal oligomers in the lens and has decreased solubility. We investigated UPR in the α B-R120G in vivo model of hereditary cataract, and found the activation of the IRE-1 branch of the UPR is activated in this model, as shown by the enhanced expression of XBP-1. We also investigated the effect of protein aggregation on autophagy. We found that autophagy was inhibited in the α B-R120G mouse lenses in vivo, as shown by the increase in number and size of p62-containing puncta and an increase in the size of autophagosomes. These findings indicate that novel pathways regulate the pathogenesis of hereditary cataracts.

IRB Status: Approved

Disclosures:

USHA ANDLEY, PHD: No financial relationships to disclose

0034

INTEGRATED PROTEOLYTIC CONTROL IN LENS AND RETINA BY UBIQUITIN, AUTOPHAGIC, AND CALPAIN SYSTEMS

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The lens and retina are metabolic opposites. Protein turnover in the lens is measured in decades, whereas turnover in the

retina is among the fastest in the body. There are two systems for removing damaged or obsolete proteins: the cytoplasmic ubiquitin proteolytic system (UPS) and the lysosomal/autophagic pathways. In the inner fiber cells of the lens options for turnover are limited because most organelles, including lysosomes, are destroyed. In lens, we found that expressing a ubiquitin mutant, K6W-Ub, alters UPS function, perturbs gap junction function resulting in Ca²⁺ elevation, hyperactivation of calpain and associated cleavage of substrates, culminating in developmental defects and a cataractous lens. The data show novel connections between UPS and calpain-based degradative systems and advance our understanding of roles for Ub K6 in eye development.

RPE must degrade enormous quantities of debris derived from the photoreceptors outer segments that are shed daily. Higher glycemia diets adversely affect both lens and retinal function in human and mammalian model systems (1). UPS and lysosomal/autophagic systems are engaged in removal of glycatively modified proteins from RPE (2).

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1. Chiu et al. Informing food choices and health outcomes by use of the dietary glycemic index. *Nutr Rev.* 2011 Apr;69(4):231-42. PMID:MC3070918

2. Uchiki et al. Glycation altered proteolysis as a pathobiologic mechanism that links dietary glycemic index, aging, and age related diseases in non diabetics. *Aging Cell.* 2012;11:1-13. PMID:PMC3257376

IRB Status: Approved

Disclosures:

ALLEN TAYLOR, PHD: No financial relationships to disclose

0035

CHROMATIN STRUCTURE AND DYNAMICS AND THEIR ROLES IN LENS FIBER CELL DENUCLEATION

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ALBERT EINSTEIN COLLEGE OF MEDICINE¹; ALBERT EINSTEIN COLLEGE OF MEDICINE, GENETICS²; FIRST FACULTY OF MEDICINE, CHARLES UNIVERSITY, PATHOLOGICAL PHYSIOLOGY³; ALBERT EINSTEIN COLLEGE OF MEDICINE, CELL BIOLOGY⁴

Lens fiber cell differentiation is characterized by highly coordinated degradation of mitochondria and nuclei. The key enzyme executing the denucleation procedure is an acidic endonuclease DNase IIb/DLAD. Down-regulation of multiple DNA-binding transcription factors, including Foxe3 and Hsf4, disrupts expression of DNase IIb, and, hence inhibits the denucleation. Chromatin structure of the 30 nm fiber and its folding requires linker histone H1 proteins. ATP-dependent chromatin remodeling complexes (SWI/SNF, ISWI, INO80, SWR1 and NuRD) employ different catalytical subunits to

induce local chromatin conformational changes including removal of nucleosomes to activate and repress gene expression. The Brg1/Smarca4 and Snf2h/Smarca5 are enzymes of the SWI/SNF and ISWI complexes, respectively. The Brg1/Smarca4 and Snf2h/Smarca5 enzymes are also involved in DNA repair. Six linker histone variants, H1⁰, H1a, H1b, H1c, H1d and H1e are expressed in somatic cells. Conditional inactivation of Brg1 and Snf2h in lens progenitor cells, mediated by le-cre, resulted in disrupted lens fiber cell differentiation including retention of the nuclei. RNA expression profiling revealed that Brg1 and Snf2h mostly regulate distinct sets of genes. Nevertheless, down-regulation of Hsf4 and Dnase2b was found in both systems. In addition, reduced expression of Foxe3 was found in Snf2h mutated lenses. Chromatin immunoprecipitations (ChIPs) identified Brg1 and Snf2h at the Foxe3, Hsf4 and Dnase2b transcriptional regulatory regions. The Snf2h mutated lenses display additional defects related to the regulation of cell cycle exit and differentiation of the lens epithelium. H1⁰ is the prevailing H1 variant in lens fiber cells. Depletion of H1 histones also obstructs the denucleation process. These studies demonstrate that chromatin structure and dynamics are critical for the process of nuclear degradation in terminally differentiated lens fiber cells. The presence of nuclei and other organelles generates scattered light incompatible with the optical requirements for the lens.

IRB Status: None

Disclosures:

ALES CVEKL, PHD: No financial relationships to disclose

0036

REPAIR CELL ACTIVATION IN RESPONSE TO LENS DYSMORPHOGENESIS CAN INDUCE PROPERTIES ASSOCIATED WITH FIBROSIS

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Previous studies have identified a subpopulation of cells of mesodermal origin within the lens epithelium that play a dramatic role in directing repair. These vimentin-rich repair cells have the ability to rapidly emerge from niches and expand their population upon injury, yet also have the potential to differentiate to a myofibroblast phenotype expressing α -Smooth Muscle Actin (α -SMA) and assembling it into an actin-stress-fiber cytoskeletal network, causing fibrotic disease. Our studies revealed that upon 24-hour treatment of E10 chick embryo lenses in organ culture with high dose (1 μ g or 10 μ g) nocodazole, there was evidence of fiber cell dysmorphogenesis and separation of the lens epithelium and fiber cells along the epithelial-fiber cell interface (EFI). Within the sites of separation, populations of vimentin-rich repair cells emerged. These cells were found to express collagen type I, well known to be an early component of an altered extracellular matrix environment that induces differentiation to a myofibroblast phenotype. These studies suggest that repair cells are activated in response to lens dysmorphogenesis, similar to the mechanism of repair cell activation following mock

cataract surgery and that they have the potential of creating lens opacities if induced to differentiate to myofibroblasts, as occurs in Posterior Capsular Opacification.

IRB Status: None

Disclosures:

CAITLIN LOGAN, BA: No financial relationships to disclose

LE07 – Lens Development

0037

THE STORY OF LENS: REGENERATION AND EVOLUTION

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The lens of adult newts can be regenerated throughout their lives. This remarkable phenomenon occurs via transdifferentiation of the dorsal iris pigment epithelial cells. The ventral counterpart is not capable of regeneration and this difference has sparked intense research into the mechanisms of induction of lens regeneration. Recent studies on gene expression patterns using transcriptomic analysis have pinpointed genes that might be responsible for regeneration. Also other studies on induction had shown that manipulation of the six-3 and BMP pathways could induce the ventral iris. In regards to lens evolution we have studied gene expression patterns in developing Nautilus eye (pinhole eye, no lens) and in pygmy squid (camera type eye with lens). We found that while both eyes express pax-6, Nautilus does not express six-3 or its downstream targets. Thus these different aspects of lens biology indicate that the six-3 pathway is crucial for the acquisition of lens.

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IRB Status: None

Disclosures:

PANAGIOTIS TSONIS, PHD: No financial relationships to disclose

0038

LENS DEVELOPMENT IN ZEBRAFISH

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Zebrafish have become increasingly useful as animal models of human visual system disorders owing largely to the ability to perform forward genetic screens and rapidly identify the affected loci. With an interest in lens development and cataractogenesis, we have recently completed a forward genetic screen and identified numerous recessive zebrafish mutations that disrupt lens formation, lens fiber organization/maintenance and lens transparency (Lee et al., 2013, IOVS). Here, we have utilized a whole-genome sequencing and SNP mapping approach to rapidly clone the affected loci. Results of the screen and gene identification will be the focus of this

presentation; several of the mutants will be described in detail, and the roles of the affected gene products during normal lens development will be discussed.

IRB Status: None

Disclosures:

JEFFREY GROSS, PHD: No financial relationships to disclose

0039

SPECIFICATION OF NEURAL RETINA IDENTITY BY LENS-DERIVED BMP SIGNALING

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The formation of the eye has served as a classical model to study tissue induction for over a century. Nevertheless, how prospective lens cells and retinal progenitors interact with and regulate each other's development remains controversial. In addition, the molecular mechanisms specifying retinal character in relation to different forebrain territories have been poorly characterized. During the early development of the vertebrate central nervous system (CNS), the anterior neural domain becomes restricted into different regions giving rise to the telencephalon, the optic cup and the diencephalon. Simultaneously, cells contributing to the peripheral nervous system (PNS), including lens placodal cells, are specified in the neural plate border (border). Thus, the formation of the eye involves at least two critical questions; i) how is the optic cup, giving rise to retinal cells, and other forebrain structures differentially specified, and ii) how does the retina, which belongs to the CNS, and the lens, belonging to the PNS regulate the development of each other. We have analyzed these issues by using intact chick embryo and explant assays. We find that BMP signaling is sufficient to replace the capacity of lens ectoderm to induce neural retina specific gene expression. In addition, our results provide evidence that BMP signaling specifies neural retina character at the expense of telencephalic cell identity.

IRB Status: None

Disclosures:

TANUSHREE PANDIT, PHD: No financial relationships to disclose

0040

A NETWORK OF GROWTH FACTOR SIGNALING DETERMINES LENS POLARITY AND REGULATES ITS PRECISE THREE-DIMENSIONAL CELLULAR ARCHITECTURE

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UNIVERSITY OF SYDNEY, SAVE SIGHT INSTITUTE¹; UNIVERSITY OF SYDNEY, BOSCH INSTITUTE²

How the lens generates and maintains its polarised three-dimensional cellular architecture is a poorly understood part of its developmental program; yet, precise regulation of this

characteristic feature is critical for function. During lens morphogenesis cells become organized into a polarized, spheroidal structure with a monolayer of epithelial cells overlying the apical tips of elongated fiber cells. During growth, epithelial cells proliferate and progeny that shift below the lens equator elongate into new fibers that become polarized/oriented towards the epithelium and undergo directed migration to the poles. This study set out to investigate interactions between epithelial cells and fibre cells in order to better understand mechanisms that underlie their assembly into a polarised spheroidal structure. Epithelial explants from neonatal rats were treated with 200 ng/ml FGF2 to promote fibre differentiation. After 4-6 days, epithelial and fibre cells were identified by immunofluorescent localization of cell-specific markers. Components of Wnt-Frizzled and Notch/Jagged signaling pathways were identified by immunofluorescence and Western blotting. Using confocal microscopy, we showed that polarized/oriented behavior of elongating fibers in FGF-treated explants is coordinated by islands of residual epithelial cells and provide evidence that this is mediated by an epithelial-derived Wnt. We also show that a reciprocal interaction occurs whereby elongating fibers, by means of a Jagged/Notch signaling pathway, promote proliferation of the associated epithelial cells and maintenance of the epithelial phenotype. This study identifies mechanisms, intrinsic to the lens, that play key roles in regulating self-assembly of epithelial and fiber cells into their distinctive polarized arrangement.

IRB Status: International

Disclosures:

JOHN MCAVOY, PHD: No financial relationships to disclose

O041

DNA METHYLTRANSFERASES IN MOUSE LENS DEVELOPMENT

MICHAEL ROBINSON, Thanh Hoang, Devin Bruney, Savana Rosalez, Blake Rasor, Blake Chaffee, Evan Horowitz

MIAMI UNIVERSITY, DEPARTMENT OF BIOLOGY

Both the specification of the lens from the embryonic surface ectoderm and the subsequent differentiation of lens fiber cells from lens epithelial cells require the induction and repression of specific gene expression. The relatively recent realization that much of the control of gene expression relies on the epigenetic state of chromatin prompted the current investigation concerning the developmental function of DNA methylation during mouse lens development. Three DNA methyltransferases catalyze the methylation of cytosine in the context of a CpG sequence in mammalian DNA. These are DNMT1, which exhibits a preference for hemi-methylated DNA and DNMT3a and DNMT3b which are known as de novo DNA methyltransferases. Immunohistochemical analysis demonstrated that DNMT1 protein primarily associates with the lens epithelium and decreases in abundance as the lens progresses through postnatal stages. In the newborn lens, Dnmt3a transcripts are most abundant and preferentially expressed in the fiber cells. Dnmt1 and Dnmt3b transcripts

predominate in the lens epithelium with levels of Dnmt3b much lower than Dnmt1. *Dnmt1*, *Dnmt3a* and *Dnmt3b* were all specifically deleted during mouse eye development using either *Le-Cre* or *MLR10* Cre alleles. The deletion of *Dnmt1* with *Le-Cre* led to substantial apoptosis in the lens epithelium and delayed eyelid closure while *Dnmt1* deletion with *MLR10* resulted in variable postnatal cataracts and microphthalmia. In contrast, deletion of either *Dnmt3a* or *Dnmt3b* or both *Dnmt3a/b* failed to alter the morphological development of the lens or alter the expression level of Dnmt1 whether the deletion was catalyzed by *Le-Cre* or *MLR10*. These findings suggest that while maintaining the state of DNA methylation is essential for lens cell survival, de novo DNA methylation catalyzed by DNMT3a and/or DNMT3b is dispensable for mouse lens development subsequent to the lens placode stage.

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IRB Status: Approved

Disclosures:

MICHAEL ROBINSON, PHD: No financial relationships to disclose

O042

FUNCTION OF RNA BINDING PROTEINS IN LENS DEVELOPMENT

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Although the human genome encodes several hundred RNA binding proteins (RBPs), mutations in less than twenty have been associated with developmental defects or disease. Using the bioinformatics-based eye gene discovery tool *iSyTE* (integrated Systems Tool for Eye gene discovery, <http://bioinformatics.udel.edu/Research/iSyTE>), we have identified a conserved RBP Celf1 (CUGBP, Elav-like family member 1; also known as Cugbp1) with a critical regulatory function in vertebrate lens development. Celf1 encodes an RBP with three RNA Recognition Motifs that directly interact with target RNAs to control mRNA decay, pre-mRNA alternative splicing or translation of mRNA into protein. We find that *Celf1* deficiency in mouse or *Zebrafish* causes severe lens defects including cataracts. Besides the presence of vacuoles, germline and lens-specific *Celf1* knockout mouse mutant lenses exhibit the presence of nuclei in centrally located fiber cells, indicating a severe defect in fiber cell differentiation. RNA-immunoprecipitation (RIP) and Cross-linked immunoprecipitation (CLIP) assays demonstrate that Celf1 mediates post-transcriptional

control of gene expression in lens development by directly binding to specific lens-expressed mRNAs (e.g. *Dnase2b*) and controlling their stability or translation. Interestingly, we find that Celf1 mediates control of the cyclin-dependent kinase inhibitors p21, p27 and p57 by distinct regulatory mechanisms. This finding represents the first example of a novel RBP-mediated mechanism of controlling cell cycle regulators in a developing tissue. In light of our recent discovery of a conserved function of the Tudor family protein *Tdrd7* in human, mouse and chicken eye development (Lachke *et al. Science* 2011 331:1571-76), these findings suggest that conserved post-transcriptional regulatory circuitries have evolved to control eye development in vertebrates.

IRB Status: None

Disclosures:

SALIL LACHKE, PHD: No financial relationships to disclose

LE08 – Signaling Pathways Determining Lens Cell Fate and Differentiation

0043

SYNERGISTIC INTERACTION BETWEEN THE FGF AND BMP SIGNALING PATHWAYS IN LENS CELLS

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OREGON HEALTH AND SCIENCE UNIVERSITY

FGFs play a central role in two processes essential for lens transparency, secondary fiber differentiation and gap junction-mediated intercellular communication (GJIC). Using serum-free primary cultures of embryonic chick lens cells (DCDMLs), we have shown that the ability of FGF to enhance fiber differentiation and GJIC is dependent on signaling by BMP endogenously produced by lens cells. In this study, we used DCDMLs to investigate how the FGF and BMP signaling pathways positively cooperate to regulate lens development and function. We found that culturing DCDMLs for six days with the BMP blocker noggin inhibits the canonical FGF-to-ERK pathway upstream of FRS2 activation and also prevents FGF from stimulating FRS2- and ERK- independent gene expression, indicating that BMP signaling is required at the level of FGF receptors. Other experiments revealed a second type of BMP/FGF interaction by which FGF promotes expression of BMP target genes as well as of BMP4. Together, these studies reveal a novel type of cooperation between the FGF and BMP pathways in which BMP keeps lens cells in an optimally FGF-responsive state and, reciprocally, FGF enhances BMP-mediated gene expression. This interaction can explain why disruption of either FGF or BMP signaling in the lens leads to defects in lens development.

IRB Status: Verified

Disclosures:

LINDA MUSIL, PHD: No financial relationships to disclose

0044

PROX1 TRANSCRIPTIONAL UPREGULATION OF FIBROBLAST GROWTH FACTOR RECEPTOR EXPRESSION IS REQUIRED FOR LENS FIBER DIFFERENTIATION

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Lens Epithelial Cells (LECs) elongate while drastically altering their proteome to differentiate into Lens Fiber Cells (LFCs) at the lens equator. Aberrant LFC differentiation can result in the development of anterior subcapsular cataract or Elschnig pearl type posterior capsular opacification, a common side effect of cataract surgery. LFC differentiation requires Fibroblast Growth Factor (FGF) signaling and expression of the several transcription factors including Prox1. Although previous reports suggest Prox1's primary role in lens development is to facilitate cell cycle exit in differentiating LFCs, RNA-seq analysis of Prox1 null lenses revealed that Prox1 is likely to directly regulate the expression of most known lens fiber cell differentiation markers including crystallins and membrane markers like Aquaporin 0 and Connexin 50. These data also suggest that Prox1 is required for expression of cytoskeletal genes and regulators which may function in cell elongation. Further, these data suggest that Prox1 also regulates the expression of several Fibroblast Growth Factor Receptors (FGFR) and their activation of downstream MAPK signaling, which may enhance the response of developing LFCs to FGF. Correspondingly, prior studies have shown that FGF signaling can upregulate Prox1 expression in the lens and that Prox1 directly interacts with the FGFR3 promoter and transcriptionally upregulates its expression. Together these data suggest positive feedback of Prox1 with FGFR driving LFC differentiation, and that this process is essential for lens development.

IRB Status: Verified

Disclosures:

DYLAN AUDETTE, BA: No financial relationships to disclose

0045

FGFR-ERK SIGNALING IN LENS, CORNEAL EPITHELIUM, AND LACRIMAL GLAND DEVELOPMENT

LIXING RENEKER, Dinesh Upadhyay, Jinglin Zhang

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The lens, corneal epithelium and lacrimal glands on ocular surface are all derived from a common head ectodermal origin during vertebrate eye development. Fibroblast growth factor receptor (FGFR)-signaling has been implicated to play a critical role in the development of these tissues. Previous studies showed that FGFR2 is required for lacrimal gland induction when *Fgfr2* was conditionally deleted using a surface ectodermal Cre line (*leCre*). In *Fgfr2^{CKO}* embryos, lens induction and cell proliferation were not affected, but cell cycle withdrawal during fiber differentiation and cell survival were impaired. In contrast to the lens, we found that FGFR2 is essential for corneal epithelial cell proliferation but not

for cell survival during early eye development. One of the major downstream effectors of FGFR-signaling is the ERK1/2 MAPKs. To investigate the role of ERK1/2 in the development of these surface ectodermal derived tissues, we examined tissue induction, cell proliferation, differentiation and survival in the *leCre-ERK1/2* double deletion embryos. We found that ERK1/2 is required for lacrimal gland induction, suggesting that this process is controlled by the FGFR2-ERK1/2 signaling pathway. Deletion of ERK1/2 did not affect lens induction or cell proliferation, but disrupted normal cell cycle withdrawal, crystallin expression and survival in primary fiber differentiation. In later steps of lens development, ERK1/2-signaling is required for cell proliferation, differentiation and survival. Interestingly, although FGFR2 is essential for corneal epithelial cell proliferation, ERK1/2 are dispensable in this process, suggesting that FGFR2 functions through ERK1/2-independent pathways in corneal epithelium. Overall our data indicate that FGFR2-ERK1/2 signaling plays different roles in lacrimal gland, lens and corneal epithelial development. Other signaling activities in FGFR2^{CKO} and ERK1/2^{DKO} eyes are currently under investigation.

IRB Status: None

Disclosures:

LIXING RENEKER, PHD: No financial relationships to disclose

O046

PI3K SIGNALING IN LENS DEVELOPMENT

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PATHOLOGY AND CELL BIOLOGY

The developmental program of lens requires cooperative signaling from several pathways. Previous studies have shown that FGF signaling can be transmitted through both Ras/MAPK and PI3K/AKT pathways. Although both biochemical and genetic experiments have demonstrated that Ras/MAPK pathway is required for differentiation and proliferation of lens cells, the role of PI3K/AKT pathway is less well understood. We have generated a lens-specific knockout of p85, a regulatory subunit of PI3K. The p85 mutant lens exhibits a loss of AKT activation, but phospho-ERK level was unchanged. These signaling defects were accompanied by significant increase in lens cell apoptosis as indicated by TUNEL staining, confirming the role of PI3K-AKT signaling in lens cell survival. Our results further indicate that p85-regulated PI3K signaling regulates differentiation of lens cells. This study establishes an important function of PI3K signaling in lens development.

IRB Status: None

Disclosures:

XIN ZHANG, PHD: No financial relationships to disclose

O047

PI3K, PTEN, AND AKT IN LENS GROWTH AND HOMEOSTASIS

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Signal transduction pathways not only play critical roles in lens development, but also contribute to homeostatic regulation in the mature lens. To further investigate how signaling regulates lens homeostasis, we have generated and examined mice where either the catalytic subunits (p110alpha and p110beta) of phosphoinositide 3-kinase (PI3K), or PTEN (PI3Ks antagonistic phosphatase) were conditionally deleted in the lens using MLR10 cre transgenic mice. Lenses of age-matched animals were dissected and photographed through a microscope equipped with a digital camera. Cell proliferation was quantified by labeling S-phase cells with EdU. Akt activation was examined by western blotting for phosphorylated and total Akt. Activation of Akt was decreased in either p110alpha or p110beta PI3K KO animals and elevated in PTEN KO lenses when compared to WT mice. Lens specific knockout of p110alpha resulted in a statistically significant reduction of lens size by ~30% in vivo. Conditional knockout of p110beta had no effect on lens size. Neither p110alpha, nor p110beta KO affected lens clarity. PTEN knockout lenses showed a slowly developing phenotype, increasing in size between 5 and 12 weeks, and then rupturing between 12 and 24 weeks. Double knockout of p110alpha and PTEN restored the size deficit caused by p110alpha deletion alone, but failed to rescue lens rupture caused in single PTEN KO mice. These data suggest that the PI3K signaling pathway regulates lens size and that PTEN plays an important role in maintaining postnatal lens integrity and transparency.

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IRB Status: Approved

Disclosures:

THOMAS WHITE, PHD: No financial relationships to disclose

O048

NEGATIVE REGULATORS OF GROWTH FACTOR SIGNALING IN THE LENS

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Lens cellular processes, such as epithelial cell proliferation and fiber differentiation are regulated by multiple growth factors in the eye, including FGF, that induce various intracellular receptor tyrosine kinase (RTK) signaling pathways (e.g. MAPK/ERK1/2). In turn, these signaling pathways are tightly regulated by antagonists, including members of the Sprouty (Spry), Sef and Spred families. These negative regulators are all expressed in lens during its differentiation and growth, and have been shown to

play distinct roles in lens biology and pathology. For example, deregulation of Spry leads to aberrant lens development, as well as a fibrotic response, typical of TGFβ-induced epithelial to mesenchymal transition (EMT) leading to cataract. In contrast, deregulation of Sef specifically impacted on lens fibre differentiation, similar to a deficiency in FGF receptor signaling. Given these different antagonists are thought to act on common intracellular pathways, in the lens, this isn't necessarily the case. In this study we used lens epithelial explants and genetically modified mice to modulate growth factor signaling by deregulating these antagonists, and examine for changes in lens cell behavior and lens development. We show that overexpression of Spreds in lens of transgenic mice for example, present a reduced lens size with subsequent posterior capsule rupture. The reduced lens size appears to result from a decreased rate of lens epithelial cell proliferation and/or reduced rate of secondary fibre differentiation. The ability of cells overexpressing Spreds to proliferate in vitro was also compromised in direct response to growth factors, such as FGF. Overall our findings support a distinct role for different RTK antagonists in regulating the signaling processes essential for lens development, and maintenance of its structure and function. By characterising the molecular interactions leading to specific lens signaling pathways, this may serve to better understand lens developmental processes, as well as processes leading to cataract.

IRB Status: International

Disclosures:

FRANK LOVICU, PHD: No financial relationships to disclose

LE09 – PCO: Can We Prevent Secondary Cataract?

O049

THE ROLE OF ECM IN POSTERIOR CAPSULAR OPACIFICATION (PCO)

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Fibronectin is an extracellular matrix (ECM) protein produced by the lens throughout its development. Fibronectin deposition in ECM is required for lens placode thickening and invagination. Further fibronectin expression increases during progression of posterior capsule opacification and is a marker of lens epithelium-epithelial to mesenchymal transition (EMT). However, little is known about whether fibronectin plays either a role in the latter stages of lens development or PCO. In order to investigate these questions, mice were created homozygous for fibronectin floxed allele and MLR10-Cre transgene (cFN) which is active in all lens cells starting at lens vesicle stage. Loss of fibronectin expression was confirmed by PCR and immunostaining. cFN lenses are transparent, with no morphological abnormalities when compared to control lenses. To determine role of fibronectin in regulation of fibrotic PCO, cFN mice were subjected to surgery modeling human cataract surgery. Capsular bags were then analyzed at 12 hrs, 48 hrs and five days post surgery for PCO development by analyzing multiple EMT

and fiber differentiation markers. At five days post surgery. cFN capsular bags expressed lower levels EMT markers such as αSMA, however no differences were observed in levels of fiber cell markers such as cMaf. Analysis of pSMAD 3 levels (a proxy for TGFβ signaling) at five days post surgery revealed that cFN capsular bags had lower levels of pSmad3 as compared to controls. Our data suggests that loss of fibronectin after lens vesicle formation does not affect lens development and morphology. However fibronectin appears to be crucial for TGFβ activation critical for the development of fibrotic PCO. Overall these data demonstrate a key role of fibronectin in EMT during PCO development but not lens fiber differentiation.

IRB Status: Verified

Disclosures:

MELINDA DUNCAN, PHD: No financial relationships to disclose

O050

POTENTIAL SIGNALING PATHWAYS TO TARGET IN PCO

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MCMASTER UNIVERSITY

TGFβ-induced epithelial-mesenchymal transition (EMT) is involved in the formation of anterior subcapsular cataracts (ASC) as well as posterior capsular opacification (PCO). Our previous work suggests that components of the cytoskeletal signaling pathway, including Rho-ROCK and myocardin related transcription factor A (MRTF-A), participate in the EMT of LEC. We have further shown that inhibition of the matrix metalloproteinase, MMP-9, suppresses TGFβ-induced ASC formation and this appears to occur through prevention in the EMT of lens epithelial cells (LEC). We are now exploring the relationship between actin-mediated pathways and the involvement of MMP-9. Using rat lens explants we found that MRTF-A was predominantly localized in the cytoplasm with negligible αSMA expression. Treatment with TGFβ triggered a transformation of LEC into an elongated mesenchymal phenotype, with nuclear MRTF localization and increased expression of αSMA. In contrast, LEC explants treated with TGFβ in the presence of the Rho inhibitor, Y27632, exhibited cytoplasmic MRTF-A staining with negligible αSMA expression. Results indicate that explants can be successfully transfected with adenoviral constructs and when transfected with a dominant-negative MRTF-A, DN-MRTF-A, a decrease in αSMA expression occurred, even in presence of TGFβ. Finally, lens explants from MMP-9 KO mice treated with TGFβ did not express αSMA like their wild-type counterparts and also showed altered morphology. The current findings demonstrate that inhibition of cytoskeletal signaling pathways in LEC prevents TGFβ-induced EMT. Since MMP-9 KO mouse explants are resistant to TGFβ-induced EMT and have an altered morphology we are investigating the possibility that they lack the cytoskeletal triggers required to induce EMT.

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IRB Status: None

Disclosures:

JUDITH WEST-MAYS, PHD: No financial relationships to disclose

THE ROLE OF REPAIR CELLS IN WOUND HEALING AND FIBROSIS IN A LENS CATARACT SURGERY MODEL

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Vimentin rich repair cells, an innate subpopulation of the lens epithelium, serve as the directors of the wound healing process after cataract surgery. These cells also have the potential to differentiate into myofibroblasts, the primary source of fibrotic diseases such as Posterior Capsule Opacity (PCO). Here, in our studies with an ex vivo mock cataract surgery wound model we present exciting new evidence about the factors that regulate repair cell function. Our findings include new insight into the lineage of repair cells. Vimentin-rich repair cells were found to be positive for the transcription factors pax3 and pax7, evidence of their mesodermal lineage, and distinguishing their lineage from that of lens epithelial and fiber cells. We identified the cytoskeletal elements involved in coordinating repair cell function for wound healing. The vimentin intermediate filament protein, and its association with myosin IIB, emerged as the major regulators of repair cell migration onto the wound edge and the role of these cells in modulating an effective wound response. In addition, we have identified signaling pathways that are essential to repair cell function such as FAK and pathways involved in their differentiation to myofibroblasts. The fate of repair cells and their role in fibrotic disease was found to be closely tied to their microenvironment. This included a role for mechanotransduction signaling in determination of a myofibroblast fate. The mechanisms identified here have the potential to identify novel therapeutic targets for both effective wound repair and the prevention of fibrotic disease.

IRB Status: Verified

Disclosures:

JANICE WALKER, PHD: No financial relationships to disclose

REGULATION OF LENS FORMATION AND MAINTENANCE BY PRIMARY CILIA

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The primary cilium, a microtubule-based organelle found in most cells, is a centre for mechanosensing fluid movement and cellular signaling including Hedgehog and Wnt pathways. Recently we found that each lens fibre has an apically

situated primary cilium that is polarized to the side of the cell facing the anterior pole. The direction of polarity is similar in neighboring cells so that in the global view, lens fibres exhibit planar cell polarity (PCP) along the equatorial-anterior polar axis. Since primary cilia have been shown to have a role in regulation of PCP and to act as a sensor for extracellular guidance signals, we have proposed that in lens fibres the cilia detect a global guidance cue from the anterior pole and this coordinates precise alignment/orientation of fibre cells. As part of a study to determine the function of primary cilia in lens we investigated the role of a key component of the intraflagellar transport (IFT) complex, IFT88, in cilia assembly.

We found IFT88 is expressed in lens epithelial and fibre cells. In both forms of lens cells, IFT88 co-localizes with the cilium axoneme marker, acetylated-tubulin. The lenses of orpk mice with a homozygous hypomorphic allele of IFT88 had abnormal sutures and showed defective osmotic sensitivity. No obvious histological defects were detected when IFT88 was conditionally removed from the lens after E12.5 with the MLR10 Cre line. Earlier depletion of IFT88 from the lens placode at E9.5 with the LR-Cre line caused abnormal lens formation in ~30% of the population, although defects were also observed in ~10% of control lenses. These results indicate that primary cilia may have a role during early stages of lens development to form normally shaped lenses. The primary cilia may also have a role in maintaining osmotic balance in postnatal lenses.

IRB Status: None

Disclosures:

YUKI SUGIYAMA, PHD: No financial relationships to disclose

UNDERSTANDING THE BASIS OF OPEN CAPSULAR BAG STRATEGIES IN THE PREVENTION OF PCO

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The current study aimed to evaluate the merits of an open capsular bag IOL (Anew Zephyr™) in PCO prevention. To assess the Anew Zephyr™ IOL, we employed an in vitro organ culture model using the bag-zonular-ciliary body complex isolated from human donor eyes and attached to a silicone ring. A capsulorhexis and lens extraction were performed, and an Alcon Acrysof IOL or Anew Zephyr™ IOL implanted in to the suspended capsular bags. In serum-free EMEM conditions, cell growth onto the posterior capsule (PC) of preparations implanted with an Anew Zephyr™ IOL was retarded relative to matched paired capsular bags implanted with an Alcon Acrysof IOL. We postulated that reduced growth factor availability within the capsular bag made a significant contribution to the benefits observed with the Anew Zephyr™ IOL. To test this notion we employed a non-suspended human capsular bag model in which the bag is isolated from the ciliary body. In the first instance we maintained these 'closed bag' preparations in 1.5 or 6mls of medium and observed effects on cell growth and growth factor levels in the culture medium. The rate of cell growth was reduced in the capsular bags cultured in 6mls medium, which

was also associated with reduced growth factor levels. We further adapted the capsular bag system, such that radial incisions were made in the anterior capsule, which was then folded back and secured to the dish (a fully open bag model). In serum-free conditions, growth onto the PC was limited in both 1.5 and 6mL volumes. If these preparations were maintained in the presence of 5% human serum, rapid coverage of the PC resulted. In terms of PCO prevention, open bag IOLs could provide benefit over conventional designs and such benefits are likely to result from dilution of available growth factors.

IRB Status: Approved

Disclosures:

MICHAEL WORMSTONE, PHD: Consultant/Advisor relationship with Anew Optics, Inc

O054

MMP INHIBITOR DELIVERY AND MODIFICATION AS A POTENTIAL MEANS OF MITIGATING PCO

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MCMaster UNIVERSITY

Posterior capsule opacification has been reported to affect as many as 30% of patients following cataract surgery, with a considerably higher incidence in the pediatric population. Various modifications to the lens material and lens shape have been used to minimize this complication but there has been relatively little change in the rate of occurrence. Recently it has been shown that inhibitors of matrix metalloproteinases can lead to decreases in the formation of anterior subcapsular cataracts. Therefore it was hypothesized that these same inhibitors or inhibitors that have an effect on processes further upstream such as inhibitors of transforming growth factor beta (TGFβ) may have an effect on posterior capsule opacification. Using poly dimethyl siloxane as a model lens material, modifications were made to determine the effect of modification with or delivery of potential inhibitors of matrix metalloproteinases on cellular changes indicative of posterior capsule opacification. Surface modification of the materials with either a TGFβ inhibitor, a tissue inhibitor of matrix metalloproteinases or a novel inhibitor of MMPs was compared with delivery of various MMP inhibitors from the silicone materials. Modification with the various MMP or TGF beta inhibiting materials was found to be successful using various analytical techniques. Prolonged delivery of the MMP inhibitor was observed over periods of days to weeks, despite the lack of optimization of the system. Cell studies with the various modified materials demonstrated a lack of fibrotic changes with lens epithelial cells following either modification with or delivery of the MMP or TGF beta inhibiting molecules. Therefore it is possible that incorporation of MMP inhibiting moieties or functional groups that inhibit TGF-beta may have a significant effect on posterior capsule opacification.

IRB Status: None

Disclosures:

HEATHER SHEARDOWN, PHD: No financial relationships to disclose

LE10 – Molecular Basis for Lens Architecture and Functional Quality

O055

CYTOSKELETAL SYNERGY IN LENS FUNCTION

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Lens fiber cells contain two membrane-associated cytoskeletal systems: beaded filaments, and a Tmod1-stabilized spectrin-actin filament network. Absence of Tmod1 in the mouse lens leads to subtle changes in the spectrin-actin network and decreased stiffness of the lens outer cortex, but no effects on lens transparency. Absence of beaded filaments (CP49-null) has no effect on the spectrin-actin network, but leads to a mild increase in lens opacity and decreased stiffness of both the outer and inner lens cortex. Combined absence of Tmod1 and CP49 leads to extensive disruption of the spectrin-actin network, and synergistic decreases in stiffness of the lens cortex, but no further effects on inner cortex stiffness or lens transparency over that of loss of CP49 alone (Gokhin et al, PLoS ONE 7(11): e48734, 2012). Immunostaining for connexins (Cx46, Cx50) showed unexpectedly that gap junction plaques are disrupted in cortical fiber cell membranes of CP49-null/Tmod1-null lenses, but not of Tmod1-null or CP49-null genotypes. This correlates with the gross disruptions of the spectrin-actin network observed only in CP49-null/Tmod1-null lenses, suggesting that the spectrin-actin network together with beaded filaments contributes to normal gap junction plaque assembly in outer cortical fiber cells. On the other hand, lens transparency as well as stiffness of inner cortical fiber cells is dominated by the contributions of beaded filaments, with little contribution from Tmod1 and the spectrin-actin network. Collectively, these data indicate complex cross-talk between lens cytoskeletal systems to determine lens fiber cell micro-domain organization and macroscopic lens functions of transparency and stiffness.

IRB Status: None

Disclosures:

VELIA FOWLER, PHD: No financial relationships to disclose

O056

CONTRIBUTIONS OF INTERMEDIATE FILAMENT PROTEINS TO LENS BIOLOGY

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The ocular lens assembles two major Intermediate Filament (IF) systems sequentially with differentiation. The Type III IF protein vimentin is the dominant IF protein of the mouse lens epithelium and differentiating fiber cells. In a seemingly

complementary manner, the Type VI Beaded Filament (BF) proteins filensin and CP49 are absent from the young mouse lens epithelium, and accumulate progressively in fiber cells as they mature, reaching a maximum at about the end of the structural elongation. Knockout studies establish that Beaded Filament proteins are required not to achieve the differentiated fiber cell phenotype, but are required to maintain it with age, and contribute to lens mechanical properties and lens shape. This work established that structural order per se is not a requirement for optical clarity. Recently, BF proteins have been identified in lens epithelium, but only in mice older than 5-6 weeks of age. These proteins assemble into a non-filamentous, tubular structure, which is found at a rate of about 1/cell. Both Type VI proteins are required for assembly of this structure, suggesting obligatory heteropolymerization as seen in the Beaded Filaments of fiber cells. The structure is not membrane-bound, and does not co-localize with the centrosome. It co-localizes with actin, but is negative for both tubulin and vimentin. However, in the absence of vimentin the structure changes shape, suggesting a functional interaction between these different classes of IF. Other roles for vimentin have been more difficult to define, both in lens and systemically, with the vimentin knockout routinely described as lacking any phenotypic change. We report that the absence of vimentin results in changes in lens size and density, as well as mechanical properties. Atomic Force Microscopy was used to demonstrate that vimentin contributes substantially to the mechanical properties of fiber cells, and thus to the lens as a whole.

IRB Status: International

Disclosures:

PAUL FITZGERALD, PHD: No financial relationships to disclose

O057

LENS FIBER CELL MATURATION INVOLVES UNIQUE CELLULAR PATTERNS AND SUBCELLULAR STRUCTURES

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The observation of a remodeling zone (RZ) only 40 μm wide in human lenses (Lim et al. IOVS 2009) raised many questions about the details of fiber cell differentiation within the outer cortex less than 150 μm from the lens surface. An ultrastructural analysis (Costello et al. EER 2013) showed the presence of three distinct regions within the RZ including initial formation of extensive ball-and-socket interdigitations, marked decrease in cytoplasmic texture along with increased cytoplasmic density and remodeling of the membranes to yield the characteristic undulating patterns. Recently, it was confirmed that non-human primate lenses displayed a similar RZ (Costello et al. ARVO2014). In this study other species were examined to determine whether analogous transitions were present in the outer cortex that have not been previously recognized. The outer cortical region from specimens preserved and examined

in previous ultrastructural studies were re-examined carefully. It was observed that diagnostic features of the RZ could be recognized in several species including rat, mouse, bovine and chick. There were significant differences among species and none of the non-primate lenses showed the transitions confined to such a narrow band as in humans. Undulating membranes were not found until after the RZ and, importantly, the cellular changes did not appear to involve the formation of defect structures, such as multilamellar bodies or autophagic vacuoles, or the formation of the refractive index gradient, which occurred deeper in the cortex. These cellular processes seemed to proceed simultaneously under separate regulation. The cellular transformations occurring across the RZ may represent a general phenomenon.

IRB Status: Verified

Disclosures:

MARTIN COSTELLO, PHD: No financial relationships to disclose

O058

LENS FUNCTION RESULTS FROM THE PERFECT FUSION OF CELL SHAPE AND THEIR GROWTH

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The growth and form of lens cells determine lens function. This tissue epitomizes the D'Arcy Wentworth Thompson approach of applying mathematical principles to describe and understand shape and form in living systems. The single layer of epithelial cells and their legion progeny of fibre cells constitute the lens. Depending on the animal, lenses range in size from that of a pinhead in the scallop to tennis ball dimensions in the giant squid. How can we describe such diversity in size and at the same time preserve functional nuances? My lab approaches this question across the length scales; from the nanometer scale of the assembly of key cytoskeletal components such as intermediate and beaded filaments (vimentin, BFSP1 and BFSP2 respectively), protein chaperones (CRYAB, CRYAA) and integral membrane proteins (eg AQP0) and their influence on the micrometer scale of lens cells ending finally with the millimeter dimensions of the lens itself. To appreciate the cellular organization of the lens, we have to consider the lens epithelium and its basement membrane as well as the lens fibre cells and how they establish and maintain the optimal packing arrangements to form the lens. These parameters are dependent upon many factors, but include integral membrane proteins, the cytoskeleton and the lens crystallins. The impact of changing/disrupting these compartments upon lens shape and cellular organization to extract and define the parameters that are needed to engineer a lens from scratch will be explored using ionizing radiation, lens regeneration and hardcore biochemistry. Our studies change cell dynamics in the lens epithelium and from changes in cell and epithelial metrics determine the key parameters need to form a lens from the

cytoskeleton and their associated crystallins and interaction with AQP0 to cell proliferation and regenerative capacity. Will “growth” and “form” suffice for the lens?

IRB Status: International

Disclosures:

ROY QUINLAN, PHD: No financial relationships to disclose

O059

THE DECORATION OF FIBER CELL MEMBRANE CYTOSKELETAL-LINKED DOMAINS AND THEIR ROLE IN LENS CYTOARCHITECTURE AND TENSILE PROPERTIES

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The transparency and function of the vertebrate lens depends on the precise hexagonal geometry, ordered packing, deformability, and membrane organization of fiber cells. While the cortical cytoskeletal network and junctional plaque proteins are considered to play a crucial role in these characteristics of fiber cells, the identity and role of scaffolding proteins which regulate adhesive complexes, and organization of the membrane cytoskeletal network and channel protein subdomains, remain elusive. Our recent studies revealed that Periaxin, a PDZ domain protein is not only distributed in a lens fiber specific manner localizing to the plasma membrane, but it is also co-immunoprecipitated with Ankyrin-B (AnkB), NrCAM and spectrin. In a reciprocal analysis, periaxin and NrCAM, spectrin was present in the co-immunoprecipitates of AnkB in the lens lysates. Additionally, Periaxin and AnkB showed a strong spatial overlap based on colocalization analysis. Importantly, mouse lenses derived from the periaxin null mutation and AnkB heterozygous for null mutation, reveal extensive disruptions in fiber cell hexagonal shape and radial alignment, and in the membrane organization of β -dystroglycan, connexin-50, NrCAM, ponsin, WAVE-2, filensin and spectrin-actin cytoskeleton. Disruptions in the membrane decoration profiles for these different integral, cell adhesive and cytoskeletal proteins were associated with impairments in membrane targeting/ clustering, and in stability and expression. Despite marked disruptions in fiber cell shape and membrane organization, both periaxin null and AnkB-/+ lenses remain largely transparent. These lenses however, exhibit significant decreases in stiffness relative to age-matched controls. Taken together, these observations uncover a close interaction among periaxin, AnkB, NrCAM, dystrophin-glycoprotein complex (DGC) and the spectrin-actin cytoskeleton, and play a key role in maintenance of lens cytoarchitecture and mechanical properties by regulating subdomain organization of membrane skeleton-linked proteins and cell adhesive interactions.

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Disclosures:

RUPALATHA MADDALA, PHD: No financial relationships to disclose

O060

SYNERGISTIC EFFECTS OF INTERCELLULAR GAP JUNCTION COMMUNICATION AND MEMBRANE-CYTOSKELETON NETWORK IN LENS TRANSPARENCY AND STIFFNESS

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The lens mainly consists of elongated fiber cells coupled with gap junction channels, formed by $\alpha 3$ (Cx46) and $\alpha 8$ (Cx50) connexin subunits, encoded by the *Gja3* and *Gja8* genes, respectively. *Gja3* mutations cause variable cataracts in humans and mice. We have demonstrated that multiple genetic factors in the C57BL/6J mouse strain background can suppress nuclear cataract formation caused by *Gja3* gene disruption. We have further identified that periaxin gene variances act as a genetic modifier that modulates the severity of nuclear cataracts in *Gja3* gene knockout lenses between the C57BL/6J (B6) strain and 129SvJae (129) strain backgrounds. Periaxin is a scaffold protein of the membrane-cytoskeleton network and its function is important for lens fiber cell organization and lens stiffness. Immunostaining data show that periaxin proteins are associated with the membrane-cytoskeleton of all fiber cells in 129 lenses but only appear in peripheral differentiating fiber cells of B6 lenses. Western blotting shows a drastic reduction of periaxin protein level in B6 lenses. Periaxin proteins are co-immunoprecipitated with many membrane-cytoskeletal proteins in 129 *Gja3* knockout lenses. These results suggest that the 129 periaxin protein functions as an abnormal scaffold protein to disrupt the membrane-cytoskeleton network of inner fiber cells, which triggers sequential events of calcium influx, calcium dependent protease activation and crystallin protein degradation to lead to a severe nuclear cataract. A rapid loss of the B6 periaxin protein eliminates its detrimental effect on the membrane-cytoskeleton network in lens inner fiber cells and suppresses cataract formation. Therefore, dysfunctions of gap junction communication and periaxin associated membrane-cytoskeleton network synergistically impair lens transparency and stiffness. Intercellular gap junction communication directly regulates the stability of the membrane-cytoskeleton network in lens fiber cells.

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IRB Status: None

Disclosures:

XIAOHUA GONG, PHD: No financial relationships to disclose

DIRECT AND INDIRECT MEASUREMENTS OF LENS MECHANICAL PROPERTIES

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Age-related changes in the mechanical properties of the lens have been cited as the primary cause of presbyopia. We have completed a series of experiments on both mouse and porcine lenses using a variety of experimental techniques selected to give insight into the role of the lens architecture in determining its mechanical behavior. Specifically, we have used spinning, compression, microindentation, shear rheometry, and microrheometry to quantify the mechanical property distribution in the young porcine lens. These various techniques give insight into the behavior of these lenses when they are respectively intact, sectioned, and homogenized. A large number of mouse lenses from animals covering a large age range have also been tested in compression.

This body of evidence clearly indicates the importance of lens architecture in determining its mechanical properties. Lenses which were sectioned for microindentation had slightly lower elastic moduli than those which were spun/compressed while intact, indicating that the sectioning process may alter lens properties. Homogenization of the lens decreased the elastic modulus by several orders of magnitude indicating the importance of lens architecture in determining its properties. Controlled treatment of homogenized lenses on a dynamic shear rheometer produced a spontaneous million-fold increase in elastic modulus. This experiment yields new insight into the spontaneous formation of pathological structures related to cataract.

The utility and limitations of each of these methods for gaining mechanistic insights into the lens' complex mechanical behavior will be discussed. Proper analysis of these measurements is essential to interpreting the contributions of lens structures to its overall mechanical properties.

IRB Status: None

Disclosures:

MATTHEW REILLY, PHD: No financial relationships to disclose

Glaucoma

GL01 – Trabecular Meshwork Pathogenesis

O062

ALTERED MECHANOBIOLOGY OF SCHLEMM'S CANAL ENDOTHELIUM IN GLAUCOMA

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Elevated intra-ocular pressure (IOP) in primary open-angle glaucoma is caused by obstruction in the conventional aqueous humor outflow pathway, likely within the juxtacanalicular tissue or inner wall endothelium of Schlemm's canal (SC). Because SC endothelium provides the only continuous barrier to conventional outflow and resides within a demanding mechanical environment, we hypothesized that the mechanobiology of SC cells is altered in glaucoma and contributes to outflow obstruction and IOP elevation. SC cells were isolated from 10 normal and 5 glaucomatous human donor eyes and characterized by a panel of functional and molecular markers. When perfusing cultured SC cell monolayers in the physiological, basal-to-apical direction, SC cells isolated from glaucomatous donors had reduced pore-forming ability compared to non-glaucomatous SC cells. By atomic force microscopy, glaucomatous SC cells had increased subcortical stiffness compared to non-glaucomatous SC cells, and there was a correlation between increased cell stiffness and reduced pore density across 6 SC cell strains. When normal SC cells were cultured on polyacrylamide gels of varying stiffness (1-34 kPa), stress fibers, focal adhesions and cell stiffness all increased on stiff versus soft substrates. Normal SC cell stiffness nearly doubled, as measured by optical magnetic twisting cytometry (OMTC). Glaucomatous SC cells, however, were more sensitive to substrate stiffness, exhibiting a 5-fold increase in cell stiffness by OMTC. Despite differences in cell stiffness, there were no differences in contractility between normal and glaucomatous SC cells as measured by traction force microscopy. qPCR revealed a substrate stiffness-dependent changes in 10 genes associated with ECM remodeling, cytoskeleton remodeling, and TGF- β 2/CTGF signaling, with glaucomatous SC cells showing elevated expression of CTGF, TGF- β 2 and PAI1 and reduced decorin relative to non-glaucomatous SC cells. Together, these data demonstrate that altered SC cell mechanobiology may contribute to SC endothelial dysfunction and outflow obstruction in glaucoma.

IRB Status: Approved

Disclosures:

DARRYL OVERBY, PHD: No financial relationships to disclose

O063

NOVEL CHARACTERIZATION AND LIVE IMAGING OF PROX-1 EXPRESSING SCHLEMM'S CANAL

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Schlemm's canal is an important structure of the conventional aqueous humor outflow pathway and is critically involved in regulating the intraocular pressure. In this study, we report a novel finding that prospero homeobox protein 1 (Prox-1), the master control gene for lymphatic development, is expressed in Schlemm's canal. Moreover, we provide a novel in vivo method of visualizing Schlemm's canal using a transgenic mouse model of Prox-1-green fluorescent protein (GFP). The anatomical location of the Prox-1⁺ Schlemm's canal was further confirmed by in vivo gonioscopic examination and ex vivo immunohistochemical analysis. Additionally, we show that the Schlemm's canal is distinguishable from typical lymphatic vessels by lack of lymphatic vessel endothelial hyaluronan receptor (LYVE-1) expression and absence of sprouting reaction when inflammatory lymphangiogenesis occurred in the cornea. Taken together, our findings offer new insights into Schlemm's canal and provide a new experimental model for live imaging of this critical structure to help further our understanding of the aqueous humor outflow. This may lead to new avenues towards the development of novel therapeutic intervention for relevant diseases, most notably glaucoma.

IRB Status: Approved

Disclosures:

TAN TRUONG, OD, MPH: No financial relationships to disclose

O064

RE-EXAMINING THE MONKEY OCULAR HYPERTENSION MODEL

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The monkey ocular hypertension model is commonly used to study the efficacy of new drugs to treat glaucoma. In this model, a laser is used to scarify a significant portion of the trabecular meshwork (TM) thus limiting outflow and inducing elevations in intraocular pressure (IOP). We have reported that the human TM becomes approximately 20 fold stiffer in glaucomatous individuals. The purpose of this study was to determine whether a compensatory change in TM stiffness

in response to chronic elevated IOP occurred in eyes that did not have molecular or cellular dysregulation. Six *Macaca fascicularis* monkeys underwent unilateral laser scarification. The IOPs of the monkeys were monitored monthly for 14 months and all monkeys continued to have increased IOPs in the lasered eye (OD) compared to the untreated eye (OS). The monkeys were euthanized and the section of unlasered TM was dissected from the treated eye. TM from the other eye (OS) served as a control. The samples from four of the monkeys were used to measure mRNA of connective tissue growth factor (CTGF) by qPCR. In all six monkeys, the unlasered TMs from the treated eye were softer than the control eye. CTGF mRNA levels were approximately 40% less in the unlasered TM. The data indicate that *normal* TM subjected to sustained, elevated IOP will compensatorily decrease the stiffness of the matrix in the TM suggesting a role for an adaptive response in modulation of matrix stiffness. The lowered expression of CTGF is consistent with this finding since lower amounts of CTGF have been shown to decrease matrix deposition in the TM.

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IRB Status: None

Disclosures:

PAUL RUSSELL, PHD: No financial relationships to disclose

O065

DECORIN, A MODULATOR OF TGF- β 2 AND CTGF SIGNALING IN THE TRABECULAR MESHWORK

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The critical risk factor for optic nerve damage in primary open-angle glaucoma (POAG), is an increased intraocular pressure (IOP) caused by an increased outflow resistance in the trabecular meshwork (TM). The molecular pathogenesis of the increased outflow resistance has not been identified, but TGF- β 2 and CTGF may be involved. Both growth factors contribute to an increased ECM synthesis and to an increased contraction rate in the TM and thereby can lead to an increased outflow resistance. In a healthy eye the growth factors are in a homeostatic balance, which seems to be disturbed in POAG. In this study we investigated the influence of Decorin (DCN), a potential endogenous antagonist of TGF- β 2 and CTGF, on the expression of growth factors, ECM and actin cytoskeleton components *in vitro* and *in vivo*. For that purpose we analyzed the DCN knockout mice in comparison to the wild-type littermates. Further we investigated the effects of DCN *in vivo* and *in vitro* on TM by RT-PCR, immunoblotting, immunohistochemistry and light microscopy. IOP was measured by tonometry. The DCN treatment led to a decreased synthesis of CTGF, TGF- β 1 and 2 in HTM cells, accompanied by a reduced expression of ECM proteins. We show that the deficiency of DCN increases IOP and leads to optic nerve damage in mouse eyes. These changes

were associated with an induction of CTGF, fibronectin and α -smooth muscle actin in the TM of the DCN deficient animals.

Our results strongly indicate that DCN is a modulator of TGF- β 2 and CTGF signaling in the trabecular meshwork, contributing to the homeostatic balance of growth factors. DCN could be a promising modifier of TGF- β 2 and CTGF signaling in POAG.

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IRB Status: International

Disclosures:

RUDOLF FUCHSHOFER, PHD: No financial relationships to disclose

O066

ROLE OF SPARC IN IOP REGULATION

DOUGLAS RHEE

CASE WESTERN RESERVE MEDICAL SCHOOL

Secreted Protein Acidic and Rich in Cysteine (SPARC) is the prototypical member of the matricellular family of proteins which are secreted non-structural proteins that are nearly ubiquitous and associated with extracellular matrix (ECM) remodeling, particularly fibrosis. In the eye, SPARC is highly expressed in the trabecular meshwork, particularly in the juxtacanalicular region, the location of the highest resistance to aqueous outflow. Alterations of trabecular meshwork ECM in the TM have been shown to change intraocular pressure (IOP) and alterations of juxtacanalicular ECM are associated with the primary pathophysiology of primary open-angle glaucoma (POAG). The role of SPARC in IOP regulation and POAG is not fully elucidated.

SPARC-null mice exhibit a lower IOP that is the result of enhanced aqueous drainage and not an artifact of central corneal thickness. SPARC-null mice have a more homogeneous outflow pattern of trabecular drainage i.e. less segmental flow. In response to physiologic stress, e.g. stretch, SPARC is one of the most highly expressed genes. In perfused human anterior segments, SPARC overexpression by viral transfection causes an elevation of IOP and an increase of collagen IV, fibronectin, and laminin in the juxtacanalicular TM. The mechanism of the ECM alterations remain elusive but does not appear to be a receptor mediated increase of transcription as there is no corresponding increase of mRNA transcripts.

Treatment of TM endothelial cells with transforming growth factor beta-2 (TGF β 2), which is highly elevated in the aqueous humor of POAG patients, SPARC is the most highly expressed protein and is specifically regulated by JNK, smad 3, and p38 signaling pathways. Increased expression of TGF β 2 in SPARC-null mice does not increase IOP implicating a critical role of SPARC in the pathogenesis of POAG.

IRB Status: Not provided

Disclosures:

DOUGLAS RHEE, MD: No financial relationships to disclose

GL02 – Glaucomatous Optic Neuropathy

O067

RADIATION PRETREATMENT, OPTIC NERVE HEAD CELLULAR RESPONSES, AND OPTIC NERVE INJURY IN AN EXPERIMENTAL RAT GLAUCOMA MODEL

ELAINE JOHNSON, William Cepurna, Tiffany Choe, John Morrison

OREGON HEALTH AND SCIENCE UNIVERSITY, OPHTHALMOLOGY

Initiation of cell proliferation is an early optic nerve head (ONH) response to elevated intraocular pressure (IOP) in both experimental rat and genetic (DBA/2J) mouse glaucoma models. Because exposure to radiation has been shown to protect the optic nerve (ON) in DBA/2J mice, we tested whether radiation exposure or another anti-mitotic therapy alters nerve injury or cell proliferation responses in an induced glaucoma model. Episcleral vein injection of hypertonic saline was used to elevate IOP unilaterally in 3 groups of rats: (1) otherwise untreated (UNT), (2) radiation treated (RAD) or (3) mitotic inhibitor 5-fluorouracil (5FU) treated, N>25/group. For all groups, the average mean and peak IOPs in injected (glaucoma model) eyes were elevated compared to respective fellow eyes (controls, $p<0.01$) and there were no significant differences in these parameters between glaucoma model groups indicating that treatment had no effect on the IOP elevation response. In glaucoma model eyes, approximately 35% of axons were degenerating and there were no significant differences in injury between treatment groups. Additionally, ON injury in glaucoma model eyes exposed to lower IOP levels (<25 mean IOP or <45 peak IOP) did not differ with treatment. Together, injury analysis indicated that there was no effect of either treatment on IOP-induced injury. Previously we showed that both the increased expression of cell proliferation genes and proliferation marker labeling were maximal in minimally injured glaucoma model ONH. Here we show that ONH cell density significantly increased only when the percentage of degenerating axons exceeded 40%. Colabeling studies are underway to identify the proliferating cells. This study indicates that in this rat glaucoma model neither radiation pretreatment nor 5FU antimetabolic therapy alter the onset or degree of ON injury due to elevated IOP.

IRB Status: Verified

Disclosures:

ELAINE JOHNSON, SCD: No financial relationships to disclose

O068

ASTROCYTE REACTIONS IN RESPONSE TO ELEVATED INTRAOCULAR PRESSURE

TATJANA JAKOBS

MASSACHUSETTS EYE AND EAR INFIRMARY

Astrocytes react to all kinds of injury to the CNS by becoming reactive. This is a complex process that involves morphological changes in the cells as well as changes in the gene expression profile of the astrocytes. In the optic nerve head, whether it con-

tains a collagenous lamina cribrosa or not, astrocytes ensheath ganglion cell axons and organize them into bundles. We asked whether an elevation of intraocular pressure (IOP) in itself leads to astrocyte reactivity in the optic nerve head. For this purpose, the anterior chamber of the mouse eye was cannulated with a glass needle attached to a saline reservoir. The IOP was raised to 30 mmHg for one hour. This treatment led to obvious morphological changes in the astrocytes three days after elevation of the IOP, characterized by a thickening of processes and an overall disruption of the normal arrangement of astrocyte processes. At the same time, the ganglion cell axons showed no signs of pathology either by light- or electron microscopy. Furthermore, retrograde and anterograde axonal transport was unaffected. The reactive changes in astrocytes were reversible and disappeared by 6 weeks after injury. This suggests that astrocyte reactivity is not harmful to ganglion cell axons per se. Rather, our results are consistent with the hypothesis that astrocyte reactivity, at least in its early stages, is a protective response aimed at supporting ganglion cell axons. Only if the insult is prolonged, like the persistent IOP elevation observed in glaucoma, or too severe, the protective response of the astrocytes is overwhelmed and the cells respond by forming a glial scar.

IRB Status: None

Disclosures:

TATJANA JAKOBS, MD: No financial relationships to disclose

O069

TARGETING NEUROINFLAMMATION TO COMBAT GLAUCOMA

GARETH HOWELL¹, Ileana Soto¹, Richard Libby², Michael Anderson³, Simon John¹

THE JACKSON LABORATORY¹; UNIVERSITY OF ROCHESTER²; UNIVERSITY OF IOWA³

Glaucoma is a multifactorial neurodegenerative disorder affecting 80 million people worldwide. Loss of retinal ganglion cells (RGCs) and the degeneration of their axons in the optic nerve are the major pathological hallmarks. Neuroinflammatory processes – inflammatory processes in the central nervous system (CNS) – have been identified in human glaucoma and in experimental models of the disease. Gene expression studies suggest transendothelial migration as a very early pathway significantly up-regulated in the optic nerve head in DBA/2J mice, an inherited mouse model of glaucoma. This results in a class of proinflammatory monocytes entering the optic nerve prior to detectable neuronal damage. A one-time X-ray treatment prevents this entry of monocytes and completely prevents subsequent glaucomatous damage in the vast majority of treated eyes. A single X-ray treatment of an individual eye, at a young age, provides that eye life-long protection from glaucoma. Detectable damage and dysfunction are prevented in these eyes, despite the continued presence of other glaucomatous stresses/signaling pathways that are not dependent on the monocytes.

IRB Status: None

Disclosures:

GARETH HOWELL, PHD: No financial relationships to disclose

MITOCHONDRIAL ENERGETIC IMPAIRMENT IN GLAUCOMA

IAN TROUNCE¹, Nicole Van Bergen¹, Vicki Chrysostomou¹, Kathryn Burdon², Shiwani Sharma², Alex Hewitt¹, Jaime Craig², Jonathan Crowston¹

CENTRE FOR EYE RESEARCH AUSTRALIA, UNIVERSITY OF MELBOURNE¹; FLINDERS MEDICAL CENTRE, FLINDERS UNIVERSITY²

In primary mitochondrial diseases oxidative phosphorylation (OXPHOS) impairment often results in the preferential demise of neurons with extended axons, with the retinal ganglion cell (RGC) being a model example. We have investigated OXPHOS function in peripheral cells of two independent cohorts of POAG subjects and found reproducible impairments of ATP synthesis. In vivo modelling using a unique mouse model with mild OXPHOS dysfunction shows that the RGCs are the first retinal neurons to display functional compromise in response to mechanical or metabolic insults. The interplay between OXPHOS dysfunction, mitochondrial turnover, and axonal transport of the organelle will be discussed from a perspective of recent advances in understanding of mitochondrial quality control defects in age-related neurodegenerative disease.

IRB Status: Not provided

Disclosures:

IAN TROUNCE, PhD: No financial relationships to disclose

0071

THE LAMINA CRIBROSA REGION OF THE OPTIC NERVE HEAD IN A SPONTANEOUS FELINE GENETIC MODEL OF GLAUCOMA

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UNIVERSITY OF WISCONSIN-MADISON, OPHTHALMOLOGY AND VISUAL SCIENCES¹; IOWA STATE UNIVERSITY, ANIMAL SCIENCES²; UNIVERSITY OF WISCONSIN-MADISON, PATHOBIOLOGICAL SCIENCES³; UNIVERSITY OF ARIZONA, AEROSPACE AND MECHANICAL ENGINEERING⁴

The lamina cribrosa (LC) region of the optic nerve head (ONH) is considered an important site for initial damage in glaucoma. Our objective was to compare morphological and biomechanical properties of the ONH in normal and glaucomatous cats. Globes were acquired from age-matched normal and *LTP2*^{-/-} cats immediately following euthanasia, following clinical characterization of glaucoma status by OCT, electrophysiology and rebound tonometry, with approval of the Institutional Animal Care and Use Committee. Optic nerves (ON) were dissected posterior to the globe and semi-automated axon counts performed by light microscopy. ONH tissues were trephined from the globe, post-fixed, osmicated and thin sagittal sections examined by transmission electron microscopy (TEM) or processed routinely for immunohistochemical labeling of paraffin sections. Unfixed globes were

chilled and submitted in PBS for biomechanical testing within 36h, with multiphoton imaging to detect second harmonic generation of collagen and two-photon emitted fluorescence of elastin. Imaging of the LC and peri-papillary sclera (PPS) was conducted under sequential pressure inflation (5, 10, 20 and 40mmHg) using a micro-optomechanical device. The average depth of the LC relative to the PPS was quantified at each inflation pressure. Inherited glaucoma in cats is associated with persistent IOP elevation; progressive loss of visual function; remodeling of the LC of the ONH; reactivity and proliferation of astrocytes, and loss of ON axons that closely resembles human glaucoma. LC beams of glaucomatous cats demonstrated more collagen crimp and greater deformability of the LC relative to normal cats. Our findings support key roles for altered LC biomechanical properties and reactive astrocytes in glaucomatous optic neuropathy. Our model provides a valuable resource for researchers studying the pathogenesis of glaucomatous optic neuropathy.

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IRB Status: None

Disclosures:

GILLIAN MCLELLAN, BVMS, PHD: Consultant/Advisor relationship with Ocular Services on Demand LLC; Consultant/Advisor relationship with Ocuscience LLC

0072

ACTIN-RICH ASTROCYTIC PROCESSES WITHIN THE OPTIC NERVE HEAD REORIENT EARLY AND SPECIFICALLY IN RESPONSE TO ELEVATED INTRAOCULAR PRESSURE

SHANDIZ TEHRANI, Elaine Johnson, William Cepurna, John Morrison

OREGON HEALTH AND SCIENCE UNIVERSITY

Optic nerve head (ONH) astrocytes may respond to elevated intraocular pressure (IOP), even prior to axonal injury. This study was designed to quantify the orientation of actin filaments within ONH astrocytic processes in response to experimental IOP elevation. IOP elevation was produced in rats by episcleral hypertonic saline injection and tissues were collected after 5 weeks. For comparison, ONHs post optic nerve transection only were collected at 2 weeks. Axonal degeneration was graded on a scale of 1-5 by light microscopy. Longitudinal sections of ONH (n≥4 eyes per group) were co-labeled with phalloidin (actin marker) and antibodies to astrocytic glial fibrillary acidic protein (GFAP) and aquaporin 4 (Aqp4), or axonal tubulin βIII (Tuj1). Confocal microscopy and FIJI software were used to quantify the orientation of filaments at different regions of the ONH. Phalloidin labeling in control ONHs showed stereotypically arranged actin filaments that localized parallel to GFAP and Aqp4 positivity. ONH Actin filament orientation was nearly perpendicular to axons (82.9° ± 6.3° relative to axonal axis), unlike the retrobulbar optic nerve (45.4° ± 28.7° relative to axonal axis, ANOVA p<0.05). With IOP elevation, ONH actin filament orientation became significantly rearranged, even in eyes with no perceivable axonal injury (i.e. 38.8° ± 15.1° in Grade 1, ANOVA p<0.05). With severe

injury (Grade 3-5), ONH actin filament orientation became more parallel to the axonal axis ($24.1^\circ \pm 28.4^\circ$, ANOVA $p < 0.05$). Actin filament orientation in the retrobulbar optic nerve was unchanged despite IOP elevation. ONH actin filament orientation in transected optic nerves was unchanged. These findings identify novel astrocytic structural changes within the ONH in response to IOP elevation, which appear to precede axonal injury. The alterations in ONH actin filament orientation may be an initiating event in the response to elevated IOP.

IRB Status: Approved

Disclosures:

SHANDIZ TEHRANI, MD, PHD: No financial relationships to disclose

GL03 – Molecular Mechanisms in Glaucomatous Neurodegeneration and Regeneration

O073

TRANSITIONING REGENERATIVE THERAPIES FROM THE BENCH TO THE CLINIC

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In degenerative diseases of the optic nerve (e.g. glaucoma and other optic neuropathies), retina ganglion cell (RGC) axons fail to regrow down the optic nerve back to their targets in the brain, and RGCs eventually die. The failure of axon regrowth, cellular survival, and regeneration of new cells is associated with the permanence of vision loss in these diseases. A number of new therapeutic avenues have been explored and validated in animal models that promote RGC survival and regeneration and even cell replacement. Here we briefly review the potential number of these novel approaches and discuss the particular challenges to translating these into clinical trials in humans, as well as potential approaches to overcoming such challenges.

IRB Status: None

Disclosures:

JEFFREY GOLDBERG, MD, PHD: No financial relationships to disclose

O074

COMBINATORIAL APPROACHES FOR OPTIC NERVE REPAIR

DIETMAR FISCHER

HEINRICH HEINE UNIVERSITY

Glaucoma is a leading cause of irreversible blindness worldwide and causes progressive visual impairment attributable to the dysfunction and death of retinal ganglion cells (RGCs).

Progression of visual field damage is slow and typically painless. Thus, glaucoma is often diagnosed after a substantial percentage of RGCs has been damaged. To date, clinical interventions are mainly restricted to the reduction of intraocular pressure (IOP), one of the major risk factors for this disease. However, the lowering of IOP is often insufficient to halt or reverse the progress of visual loss, underlining the need for the development of alternative treatment strategies. Several lines of evidence suggest that axonal damage of RGCs occurs primary at the optic nerve head, where axons appear to be most vulnerable. Axonal injury leads to the functional loss of RGCs and subsequently induces the death of the neurons. However, the detailed molecular mechanism(s) underlying IOP-induced optic nerve injury remain poorly understood. Therefore, protective strategies to prevent further axonal and subsequent soma degeneration are of great importance to limit the progression of sight loss. In addition, strategies that stimulate injured RGCs to regenerate and to overcome the inhibitory environment of the optic nerve are necessary for reconnection with central targets and functional restoration. This talk provides insights into strategies to promote axon regeneration focusing on the role of cytokines and their downstream signaling pathways. Moreover, the potential benefit of combinatorial approaches with disinhibitory treatments will be addressed.

IRB Status: Approved

Disclosures:

DIETMAR FISCHER, PHD: No financial relationships to disclose

O075

EXERCISE REVERSES FUNCTIONAL AND STRUCTURAL LOSS AFTER OPTIC NERVE INJURY: MEDIATION BY BRAIN-DERIVED NEUROTROPHIC FACTOR

VICKI CHRYSOSTOMOU, Ian Trounce, Eamonn Fahy, Jonathan Crowston

CENTRE FOR EYE RESEARCH AUSTRALIA, UNIVERSITY OF MELBOURNE

We recently reported that exercise can protect the neural retina against experimental injury, showing that daily forced exercise in aged mice significantly improved optic nerve functional recovery after pressure-induced injury and abrogated stress responses in retinal neurons, macrophages and glia. Here, we sought to further elucidate underlying cellular changes and mechanisms of exercise-induced retinal protection. At 12 months of age, C57BL/6J mice were randomly assigned to exercise or control groups ($n=30$ per group). Mice in the exercise group were subject to swimming (60 min/day, 5 days/wk) while control mice were exposed to exercise handling procedures (60 min/day, 5 days/wk). After 5 weeks, optic nerve injury was induced by acute elevation of intraocular pressure (50 mmHg for 30 min). Retinas were assessed for function using electroretinography and evaluated for cellular injury responses using immunohistochemistry. Exercise significantly ($p < 0.01$) protected against optic nerve dysfunction after pressure-induced injury, as measured by amplitudes of two inner retina-derived components of the

electroretinogram: the positive scotopic threshold response (pSTR) and the photopic negative response (PhNR). In addition to providing functional protection, exercise prevented injury-induced synaptic loss and thinning of the inner plexiform layer, as measured by PSD-95 immunolabeling. In a subgroup of exercised mice, systemic administration of a receptor antagonist to brain derived neurotrophic factor (BDNF) completely abolished the protective effect of exercise on optic nerve function after injury. These data suggest that exercise can protect inner retinal function and structure after injury and that this protection is critically dependent on BDNF signaling.

IRB Status: Verified

Disclosures:

VICKI CHRYSOSTOMOU, PHD: No financial relationships to disclose

O076

STRATEGIES FOR ENHANCING NEURONAL SURVIVAL FOLLOWING OPTIC NERVE INJURY

JOSEPH LEWCOCK

GENENTECH, INC.

Retinal ganglion cells are highly polarized neurons that project axons a considerable distance from the retina to the brain. In order to respond to axonal damage, as occurs in Glaucoma or following traumatic injury, these neurons must be able to transmit a retrograde signal to the nucleus to enable a transcriptional stress response. Work in our lab has identified dual leucine zipper-bearing kinase (DLK/MAP3K12) as an essential regulator of this response, and DLK deletion results in sustained protection of axons and cell bodies from degeneration through broad regulation of injury induced transcriptional changes. This neuronal protection is achieved through engagement of the DLK pathway in the axon proximal to the injury site which is subsequently propagated to the nucleus. DLK protein levels were found to be rapidly elevated following neuronal injury specifically in the damaged portion of the neuron, leading to the discovery that abundance of DLK protein in mammalian neurons is tightly regulated by the ubiquitin-proteasome system. Following optic nerve injury, phosphorylation based stabilization of DLK is essential to the initiation of an injury response through creating a positive feedback loop that amplifies stress signaling and allows neurons to appropriately respond to this localized external insult. Taken together, these data suggest that DLK is an essential regulator of the neuronal stress response whose function is required for axons to properly respond to injury and may represent an attractive drug target for Glaucoma and other indications.

IRB Status: None

Disclosures:

JOSEPH LEWCOCK, PHD: Employee relationship with Genentech, Inc.

O077

DISTINCT SIGNALING PATHWAYS CONTROL COMPARTMENTALIZED RETINAL GANGLION CELL DEGENERATION AFTER AXONAL INJURY

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UNIVERSITY OF ROCHESTER MEDICAL CENTER, FLAUM EYE INSTITUTE, OPHTHALMOLOGY¹; THE JACKSON LABORATORY²; UNIVERSITY OF ROCHESTER MEDICAL CENTER, NEUROBIOLOGY AND ANATOMY³; THE JACKSON LABORATORY AND HOWARD HUGHES MEDICAL INSTITUTE⁴

The MAPK family has important roles in both axonal and somal degeneration. It has been suggested that compartmentally distinct degeneration programs execute retinal ganglion cell (RGC) degeneration after axonal injury, including in glaucoma. We and others have shown that following axonal injury specific MAPKs, JNK2 and 3, are activated first in axons proximal to the site of insult and subsequently in RGC somas. Deletion of *Jnk2/3* or its canonical substrate, JUN, significantly delays RGC death after controlled optic nerve crush (CONC). In neurons an upstream activator of JNK, DLK (MAP3K12), is known to be activated after axonal injury and has been suggested to be critical for axonal and somal degeneration. *Dlk* deficiency partially phenocopies *Jnk2/3* deficiency in that it similarly protects RGCs from axonal injury-induced death. However, while *Dlk* deficiency does not prevent activation of the axonal pool of JNK, somal activation of JNK (and JUN) is prevented. These data suggest that DLK is required for sustaining JNK activation in axonally injured RGCs, but is not the primary MAP3K that initiates JNK signaling in RGC axons. This is consistent with studies showing different cellular pools of JNK have distinct modes of regulation. Thus, after axonal injury in RGCs, there appear to be two pools of JNK, a somal DLK dependent pool and an axonal pool dependent on an unidentified upstream kinase. These pools appear to serve diverse functions, with the axonal pool being important for stabilizing DLK levels in axons (Huntwork-Rodriguez et al., JCB, 2013), while the somal pool is upstream of the transcriptional activity required for RGC death. Surprisingly, neither deficiency in *Dlk* nor *Jnk2/3* protected optic nerves from degeneration after CONC, as judged by compound action potential amplitudes. These results highlight the need to identify pathways controlling axonal injury signaling, somal degeneration and axonal degeneration.

IRB Status: None

Disclosures:

RICHARD LIBBY, PHD: No financial relationships to disclose

O078

TRANSGENIC ANIMAL- AND STEM CELL-BASED APPROACHES TO STUDYING NORMAL-TENSION GLAUCOMA

JOHN FINGERT, Michael Anderson, Robert Mullins, Budd Tucker

CARVER COLLEGE OF MEDICINE, UNIVERSITY OF IOWA
DEPARTMENT OF OPHTHALMOLOGY

We have previously shown that duplication of the TANK binding kinase 1 (*TBK1*) gene causes some cases of normal tension glaucoma (NTG). A chief feature of NTG is loss of retinal ganglion cells (RGCs). The mechanism by which *TBK1* mutations cause RGC loss is unclear. However, there is evidence that links RGC loss with dysregulation of autophagy, a process by which cells isolate and digest proteins, organelles, and other materials. We have developed two key resources to study RGC loss and autophagy in *TBK1*-associated glaucoma: 1) a transgenic *TBK1* mouse that has a human *TBK1* transgene incorporated in its genome and 2) cultured RGC-like neurons produced using induced pluripotent stem cell technology from an NTG patient known to have a *TBK1* gene duplication. Using these resources, we have investigated the role of *TBK1* gene duplication and abnormal autophagy in RGC death and glaucoma. Studies of these resources show that key markers of autophagy (i.e. LC3-II) are altered due to *TBK1* gene duplication.

IRB Status: Approved

Disclosures:

JOHN FINGERT, MD, PHD: No financial relationships to disclose

O079

GENOME-WIDE ASSOCIATION STUDIES OF OPHTHALMOLOGY

DAVID MACKEY

LIONS EYE INSTITUTE

Genome-wide association studies (GWAS) have revolutionized our understanding of the underlying molecular mechanisms of several major eye diseases. Over the last year we have seen major international collaborations involved in diseases such as myopia, age-related macular degeneration and glaucoma successfully identify genetic pathways associated with disease. In addition smaller studies identified and confirmed genes associated with disease and endophenotypes (biometric measures associated with disease) as well as gene-environment interactions. Identification of new gene associations allows us to understand the mechanisms of disease, potentially identify individuals at increased risk and lead to new treatments for disease.

IRB Status: International

Disclosures:

DAVID MACKEY, MD: No financial relationships to disclose

O080

PRIMARY OPEN-ANGLE GLAUCOMA: GENOME-WIDE ASSOCIATION STUDIES

JANEY WIGGS

MASSACHUSETTS EYE AND EAR, OPHTHALMOLOGY

Recent genome-wide association studies (GWAS) have identified 5 genomic regions significantly associated with primary open angle glaucoma (POAG). The genes and regulatory elements located in these regions (*CDKN2BAS*, *SIX1/SIX6*, *CAV1/CAV2*, *TMCO1*, 8q22) have diverse biological functions. Several of the genomic regions associated with POAG are also associated with normal-tension glaucoma (NTG), the glaucoma subgroup with increased susceptibility to optic nerve degeneration. Pathway analyses using GWAS data have implicated cell cycle progression, cell adhesion, immune mechanisms, GABA metabolism and Acetyl-CoA metabolism in POAG and NTG. While these studies have identified important genes and pathways contributing to POAG and NTG, additional studies using larger datasets will be necessary to fully define the disease-related genetic architecture. Ongoing studies, using over 4000 cases and 35,000 controls (the NEIGHBORHOOD study) and meta-analyses involving cases and controls from the United States, Europe, Asia and Australia (the International Glaucoma Genetics Consortium) are likely to yield additional genes contributing to primary open angle glaucoma. SNP risk scores based on current and new genes associated with glaucoma could form the basis of clinically useful diagnostic and screening tests assessing POAG and NTG risk. Additionally, the identification of novel genes and pathways contributing to glaucoma could lead to the development of disease-specific therapeutic targets including neuro-protective therapies.

IRB Status: Approved

Disclosures:

JANEY WIGGS, MD, PHD: Grant Support relationship with NIH/NEI; Grant Support relationship with March of Dimes Foundation

O081

INTERLEUKIN-20 RECEPTOR EXPRESSION IN TRABECULAR MESHWORK AND ITS IMPLICATIONS IN GLAUCOMA

KATE KELLER

OREGON HEALTH AND SCIENCE UNIVERSITY, OPHTHALMOLOGY

Primary open angle glaucoma (POAG) is a genetically complex disease linked to multiple gene loci. The *GLC1C* locus was originally mapped to chromosome 3. In this study, we identify a non-synonymous mutation in a large POAG family in which the *GLC1C* locus was originally mapped. This mutation, T104M in Interleukin-20 Receptor-2 (IL-20R2), segregated with disease, but was not detected in 230 random POAG patients or 109 controls. The T104M missense change is predicted to impact the binding of IL-20R2 to the cytokines, IL-19, IL-20 and/or IL-24. To investigate whether IL-20R2 function is compromised, we quantitated the phosphorylation of Signal Transducer and Activator of Transcription-3 (STAT3), a downstream target, in normal

human dermal fibroblasts (HDFs) and POAG fibroblasts (pHDFs) harboring the T104M mutation using an ELISA assay. pHDFs had significantly increased basal levels of phosphorylated STAT3 (pSTAT3), but stimulation with either IL-19 or IL-20 significantly decreased pSTAT3 levels in pHDFs compared to controls. Since activation of STAT3 can induce matrix metalloproteinases (MMP), we also measured MMP activity. Using a quenched fluorogenic peptide substrate, which only generates fluorescence following cleavage by a MMP, we found that MMP activity was significantly decreased in pHDFs compared to controls after stimulation with IL-20 for 24 hours. Finally, we investigated IL-20R2 protein by confocal microscopy and immunoblotting in human trabecular meshwork (TM) cells, which are responsible for generating and maintaining intraocular pressure (IOP) in a normal range. IL-20R2 was up-regulated in response to all cytokines tested. Collectively, our study shows that a T104M mutation in IL-20R2 significantly impacts the biological function of this receptor in POAG fibroblasts as evidenced by decreased pSTAT3 levels and MMP activity in response to cytokine stimulation. Dysfunctional IL-20R2 may affect the ability of TM cells to homeostatically adjust outflow resistance and contribute to the observed elevated IOP in these patients.

IRB Status: Approved

Disclosures:

KATE KELLER, PHD: No financial relationships to disclose

O082

EXOME SEQUENCING REVEALED AN ASSOCIATION OF PRIMARY GLAUCOMA WITH MUTATIONS IN GENES RELATED TO ANTERIOR SEGMENT DYSGENESIS

QINGJIONG ZHANG, Xiaobo Huang, Xueshan Xiao

ZHONGSHAN OPHTHALMIC CENTER, SUN YAT-SEN UNIVERSITY

Genetic factors play an important role in glaucoma but the genetic defects for most glaucoma are awaited to be disclosed. Anterior segment dysgenesis is frequently associated with glaucoma. It would be interesting to know if genes responsible for anterior segment dysgenesis may also contribute to primary glaucoma when mutated. We have performed exome sequencing on 257 unrelated Chinese patients with glaucoma, including 125 with POAG and 132 with PACG. In the present study, Sanger sequencing was used to validate the variants detected by exome sequencing in ten genes responsible for anterior segment dysgenesis, including FOXE3, PITX2, FOXC1, EYA1, PITX3, PAX6, B3GALT1, COL4A1, MIR184, and FOXC2. Eight novel variants in 4 of the ten genes were detected in eight of the 257 (3.1%) patients with primary glaucoma, including three variants in *FOXC1*, *PITX2*, and *EYA1*, respectively, and five variants in *COL4A1*. Five of the eight patients had POAG while the rest three had PACG. The results suggest that a proportion of primary glaucoma may be caused by mutations in genes associated with anterior segment dysgenesis.

IRB Status: Approved

Disclosures:

QINGJIONG ZHANG, MD, PHD: No financial relationships to disclose

O083

IDENTIFICATION AND IN VIVO VALIDATION OF DELETIONS OF REGULATORY ELEMENTS IN PITX2 AS CAUSATIVE FOR DEVELOPMENTAL GLAUCOMA

MEREDITH PROTAS¹, Tim Footz², Scott Baraban¹, Michael Walter², Ordan Lehmann², Douglas Gould¹

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO¹; UNIVERSITY OF ALBERTA²

Mutations in *FOXC1* and *PITX2* constitute the most common genetic alterations in the etiology of Axenfeld Rieger Syndrome, a syndrome characterized by ocular anterior segment dysgenesis (ASD), an important class of development glaucoma. However, for approximately half of the patients screened – many by whole exome sequencing – the genetic cause remains unidentified. We propose that this failure, at least in part, reflects the importance of non-coding variants to ASD pathogenesis. We have performed whole genome sequencing of two diseased individuals from a large family with Axenfeld-Rieger syndrome and found a large non-coding deletion upstream of *PITX2* that segregates with the disease. We have identified the breakpoints of this almost 750 kb deletion which includes seven previously described putative regulatory elements of *PITX2* through reporter studies of the orthologous elements in zebrafish. We are utilizing the innovative and powerful genome-editing technology (CRISPR/CAS) to generate orthologous deletions of human patients, including our family and two other families with deletions of different subsets of these regulatory elements, in the zebrafish model system. We have validated the CRISPR/CAS method and our ability to generate these deletions and are currently raising fish to generate stable lines for morphological analysis. In summary, we have identified a putative mutation for Axenfeld-Rieger syndrome and are utilizing a novel method, genome editing in zebrafish, to demonstrate causality.

IRB Status: Approved

Disclosures:

MEREDITH PROTAS, PHD: No financial relationships to disclose

O084

GENE AND ENVIRONMENT INTERACTION IN THE ONSET AND PROGRESSION OF MYOPIA: GUANGZHOU TWIN EYE STUDY

MINGGUANG HE

ZHONGSHAN OPHTHALMIC CENTER

The Guangzhou Twin Registry, initiated in 2005, is a population-based registry of twins born between 1987 and 2000. To date, over 9700 pairs of twins, regardless of their health and medical history, were enrolled in the database using the Official Household Registry of Guangzhou City. A total of 1205 pairs of twins aged 7 to 15 years, as well as their parents, were enrolled in 2006. The twins have been examined annually since 2006 in order to investigate the onset and progression of myopia. Refractive data were collected by cycloplegic refraction and IOLMaster was used for biometric

data collection. A standard questionnaire was administered by in-person interview to estimate time spent on near work and outdoor activities. Genotype SNP data were generated using the Affymetrix Axiom chips. The genotypic and environmental data of the first-born twins were used for the current analysis. In this symposium, we will brief the major findings on gene and environment interaction on the onset and progression of myopia in this cohort of twins.

IRB Status: Approved

Disclosures:

MINGGUANG HE, MD, PHD: No financial relationships to disclose

GL05 – Animal Models of Glaucoma

O085

DEFINING AN AUTOIMMUNE MECHANISM OF GLAUCOMA IN A MICROBEAD-INDUCED DISEASE MODEL

DONG CHEN

SCHEPENS EYE RESEARCH INSTITUTE, HARVARD MEDICAL SCHOOL

Glaucoma is the most prevalent neurodegenerative disease and the leading cause of irreversible blindness. Although intraocular pressure (IOP) is a known risk factor, the underlying cellular and molecular mechanisms through which elevated IOP leads to neuronal damage remain unknown. Study of the pathogenesis of glaucoma has long been hampered by the lack of a suitable mouse model that would allow genetic dissection of the underlying cellular and molecular mechanisms. Recently, we and others have developed a convenient method to transiently and reversibly induce ocular hypertension in mice by injecting polystyrene microbeads into the anterior chamber of the eye. This model mimics key clinical features of primary open angle glaucoma, and allows identification of subsequent events induced by the initial IOP elevation. We show further that this model is suitable for preclinical testing and screening of ocular hypotensive drugs which lower IOP via reducing aqueous humor production. Coupled with spectrum domain-optical coherent tomograph (SD-OCT), the model allows evaluation non-invasively retinal axon damage in the glaucoma mouse model. More importantly, using this method we define a functional link between elevated IOP and the induction of autoimmune responses in the pathogenesis of glaucoma and show that transient elevation of IOP induces prolonged and progressive neurodegeneration even after the IOP returns to a normal level by stimulating an autoreactive CD4⁺ T cell response. These findings suggest novel approaches for preventing and limiting neurodegeneration and vision loss in glaucoma.

IRB Status: None

Disclosures:

DONG CHEN, MD, PHD: No financial relationships to disclose

O086

AN INDUCIBLE MOUSE MODEL OF PIGMENT DISPERSION SYNDROME

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Pigment dispersion syndrome (PDS) is an important glaucoma risk factor. PDS can lead to elevated intraocular pressure (IOP). For unknown reasons, many, but not all, eyes afflicted with PDS develop IOP elevation. In order to study the pathogenic events by which pigment causes IOP elevation, an inducible animal model could confer several advantages. Previous studies of PDS with animal models have utilized cynomolgus monkey eyes infused with pigment particles. Surprisingly, despite the experimental advantages of an inducible model such as this, there have been no reports of attempts to move the model into other species. Therefore, we have developed methodology for similarly inducing pigment dispersion in mice. In studies to date, cohorts ($n=5$ mice each) of C57BL/6J (B6) mice homozygous for the *Tyr^{c-2J}* allele (B6.*Tyr^{c-2J}/J*) have received intraocular infusion of donor iris preparations made from dissected irides of B6, B6.*Tyr^{c-2J}/J* mice, or vehicle only control. Ocular phenotypes of recipient mice were collected pre- (baseline) and post-infusion (response) using slit lamp and gonioscopic imaging, rebound tonometry to measure IOP, and histology. Infusion of recipient eyes with donor iris preparations results in anterior chamber phenotypes resembling PDS without overt signs of inflammation. Slit lamp photography illustrates that iridial melanin localizes to the tissues at the iridocorneal angle. Histological analyses show a sustained presence of pigment in macrophages and throughout the trabecular meshwork. Recipient eyes infused with iris preparations from *Tyr^{+/+}* donors exhibit significant IOP elevation ($p<0.02$), whereas preparations from *Tyr^{c-2J}* do not. These results demonstrate that ocular phenotypes of PDS can be recapitulated in this inducible mouse model. The presence of melanin in the infused iris preparations is critical for IOP elevation. In ongoing work, this inducible model is being used to manipulate features of the donor pigment and recipient tissues to study biological responses influencing pigment-induced IOP elevation.

IRB Status: None

Disclosures:

MICHAEL ANDERSON, PHD: No financial relationships to disclose

O087

INVESTIGATION OF PATHOGENIC PATHWAYS OF GLAUCOMA IN AN INDUCIBLE MOUSE MODEL SYSTEM

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The trabecular meshwork (TM) is involved in the outflow of aqueous humor and intraocular pressure (IOP) regulation. TGF- β signaling pathways in the ECM of the TM have been

extensively studied. Recent evidence has implicated toll-like receptor 4 (TLR4) in the regulation of ECM and fibrogenesis in liver, kidney, lung and skin. We investigated whether the TLR4 signaling pathway is involved in the regulation of the ECM in the TM and ocular hypertension. Human donor eyes, primary TM cells, and mouse TM were used to determine TLR4 expression in the TM. Human TM cells were treated with a selective TLR4 inhibitor (TAK-242, 15 μ M) in the presence or absence of TGF β 2 (5ng/ml). A/J, AKR/J, BALBc/J, C3H/HeJ, and C3H/HeOuj (n=5-13/strain) mice were injected intravitreally with Ad5.hTGF β 2^{226/228} in one eye, with the uninjected contralateral eye serving as a control. TLR4 was expressed in mouse (n=3 mice/strain) and human TM (n=8). TGF β 2 treatment increased fibronectin (FN) (4.46 \pm 0.07 fold) and collagen 1 (COL1) (11.53 \pm 0.41 fold) mRNA expression in primary TM cells. Inhibition of TLR4 in the presence of TGF β 2 decreased expression of FN (1.15 \pm 0.18 fold) and COL1 (4.35 \pm 0.85 fold) (n=3/treatment, p<0.001). Inhibition of TLR4 in the presence of TGF β 2 also decreased FN protein expression (0.88 \pm 0.02 fold; n=3) compared to cells treated with TGF β 2 alone (4.05 \pm 0.59 fold; n=3 p<0.01). Ad5.hTGF β 2 induced ocular hypertension in A/J, AKR/J, and BALBc/J mice throughout an eight week time course. IOPs increased to approximately 25 mm Hg in all 3 mouse strains (p<0.05). Ad5.hTGF β 2^{226/228} did not induce ocular hypertension in *Tlr4* mutant C3H/HeJ mice, but *Tlr4* wildtype C3H/HeOuj mice developed ocular hypertension to approximately 20 mm Hg (p<0.01). These studies identify TGF β 2 – TLR4 crosstalk as a novel pathway involved in ECM regulation in the TM and ocular hypertension.

IRB Status: None

Disclosures:

COLLEEN MCDOWELL, PHD: No financial relationships to disclose

O088

LONGITUDINAL CHARACTERIZATION OF LOW-TENSION GLAUCOMA IN MICROFIBRIL-DEFICIENT MICE

RACHEL KUCHTEY, Monique McCallister, Jessica Kunkel, John Scichilone, John Kuchtey

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To test the hypothesis that microfibril deficiencies cause glaucoma, mice carrying mutations in *Fbn1* gene encoding Fibrillin-1, the principal component of microfibrils were followed longitudinally for glaucoma phenotypes. Comparisons were made between 2 lines of mice heterozygous for *Fbn1* mutations (*Fbn1*^{C1039G/+} and *Tsk*^{-/-}) and homozygous normal contemporary littermate controls. Outflow facility was measured by constant pressure perfusion of enucleated eyes. Intraocular pressure (IOP) was measured in isoflurane anesthetized mice using the TonoLab instrument. Central corneal thickness (CCT), retinal nerve fiber layer (RNFL) thickness and axial length (AL) were determined by spectral domain optical coherence tomography (SD-OCT). Retinal ganglion cell (RGC) numbers were determined by counting Brn3a-stained retinal whole mounts. Although microfibril deficient mice had lower outflow facility as compared

to controls (p<0.05), their IOP was not elevated. CCT of microfibril deficient mice measured at 3 months and 1 year of age were >20% thinner than controls (p<0.0001). Differences in CCT did not affect calibration of the tonometer. Glaucomatous RGC degeneration was apparent in microfibril deficient mice as evidenced by lower RGC counts in *Tsk*^{-/-} mice by 10 months of age (p<0.05). RNFL in *Fbn1*^{C1039G/+} mice was unchanged at 3 months but thinner than control by 12- 16 months (p<0.01). AL was not significantly different between microfibril deficient and control mice. Results from these experiments with 2 separate lines of mice carrying mutations in *Fbn1* suggest that microfibril deficiencies cause glaucoma. Lower RGC counts and thinner RNFL in microfibril deficient mice in the absence of elevated IOP suggests that microfibril deficient mice may be a very useful mouse model of low tension glaucoma.

IRB Status: None

Disclosures:

RACHEL KUCHTEY, MD, PHD: No financial relationships to disclose

O089

CONTINUOUS MEASUREMENT AND CONTROL OF INTRAOCULAR PRESSURE IN RATS

CHRISTOPHER PASSAGLIA, Simon Bello, Xiaolan Tang, Sharad Malavade

UNIVERSITY OF SOUTH FLORIDA

In order to understand the pathophysiology of glaucoma, researchers employ animal models in which intraocular pressure (IOP) is elevated by experimental means. While much has been learned from these glaucoma models about the effects of high IOP on the eye, research progress remains hampered by long-standing issues with available pressure-elevation methods, such as finite success rates, sporadic IOP data, and variable IOP histories. To overcome these issues, we have developed a novel system for monitoring and regulating IOP. The system consists of a pressure sensor, microcontroller, and miniature pump that connect to the eye via a fine cannula. Bench performance of the system was evaluated by exposure to constant hydrostatic pressure for several months. Pressure was recorded with 0.2 mmHg resolution and fluctuated <1 mmHg. A surgical procedure was devised to implant the cannula in the anterior chamber of rat eyes. The procedure has high success rate, with cannula placements lasting over a year even though the eye can freely rotate. Moreover, little-to-no signs of infection, internal damage, tip clogging, or fluid leakage are presented. The system was tested on rats under ketamine-xylazine anesthesia for 1-2 days. In open-loop mode (pressure measurement only), a circadian rhythm in IOP was recorded with peak-to-peak amplitude of ~10 mmHg. In closed-loop mode (autonomous pressure control), IOP was raised and lowered to set points that ranged from 10-50mmHg and maintained within \pm 3 mmHg of the specified level for however long desired. Integrating pump output then gave the aqueous outflow rate for each IOP setting. In sum, we show that the rat anterior chamber can be cannulated without damaging the eye physically or electrophysiologically and that through the cannula

rat IOP can be continuously measured and programmatically controlled. We conclude with initial findings from the system connected to the eye of awake rats.

IRB Status: Approved

Disclosures:

CHRISTOPHER PASSAGLIA, PHD: No financial relationships to disclose

O090

GENETIC VARIANTS OF GENE *SIX6* INCREASE POAG RISK, REDUCE RNFL IN POAG CASES, AND REDUCE OPTIC NERVE VOLUME IN THE ZEBRAFISH MODEL

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Purpose: To identify sequence variants within and near the *SIX6* gene, to assess their association with primary open angle glaucoma (POAG), and to perform functional testing using the zebrafish model.

Methods: Meta-analysis of the NEIGHBOR and GLAUGEN datasets identified a region on chromosome 14q23 that is associated with POAG risk (top SNP rs10483727, p-value=3.9 X 10⁻¹¹, OR=1.32) as well as POAG quantitative endophenotypes involving optic nerve measurements. This locus contains the homeobox gene *SIX6*, which is known to play a role in ocular development. Sequence analysis identified multiple nonsynonymous coding variants that we functionally tested: morpholino antisense oligonucleotides for targeted knockdown have been injected into developing zebrafish embryos, and missense variants have been used to rescue the observed ocular phenotypes. We then analyzed automated measures of retinal nerve fiber layer (RNFL) thickness using sdOCT in POAG patients with *SIX6* risk variants.

Results: A common *SIX6* missense variant rs33912345 (Asn-141His) identified in a meta-analysis of the NEIGHBOR GLAUGEN dataset was highly associated with POAG (p-value=4.2 x 10⁻¹⁰, OR=1.54). Knockdown of *SIX6* expression in the developing zebrafish model results in significant reduction in eye size and optic nerve volume (p<0.03)(p<0.0001). The non-risk allele rs33912345 (Asn141) fully rescues the reduction in the volume of the optic nerve, while the risk allele (His141) does not (p < 0.001). RNFL, measured by sdOCT, was reduced in POAG cases homozygous for the His142 risk variant compared to cases homozygous for the Asn141 nonrisk allele (p<0.03).

Conclusions: Risk variants in the *SIX6* gene are associated with POAG. These variants are functionally significant in the zebrafish model. POAG patients who are homozygous for the common rs33912345 *SIX6* variant have reduced RNFL thickness compared to those who have the non-risk variant. The mechanism by which *SIX6* variants increase risk of POAG remains to be determined.

IRB Status: Approved

Disclosures:

R. RAND ALLINGHAM, MD: No financial relationships to disclose

GL06 – Biomechanics in Glaucoma: Trabecular Meshwork and Optic Nerve Head

O091

OCULAR BIOMECHANICS IN THE ANTERIOR AND POSTERIOR SEGMENTS

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IOP reduction is the only clinically proven means to prevent the onset and progression of glaucoma. However, we have an incomplete understanding of the factors that control IOP and how IOP causes the retinal ganglion cell axon damage at the optic nerve head. This presentation will introduce and summarize current concepts in ocular biomechanics related to glaucoma. In the anterior segment, the trabecular meshwork functions as the primary drainage route for aqueous humor, and increased trabecular outflow resistance causes IOP elevation. Therefore, understanding the biomechanical basis of trabecular outflow regulation will provide insight into the pathogenesis of glaucomatous ocular hypertension and identify new strategies to restore homeostatic IOP levels by targeting trabecular outflow function. In the posterior segment, the peripapillary sclera and optic nerve head (ONH) define the biomechanical insult that contributes to glaucomatous blindness. Therefore, understanding the material properties and morphology of the ONH and peripapillary sclera, and how these tissues sense and respond to IOP, will reveal the pathological mechanisms leading to glaucomatous blindness and identify new strategies to protect the ONH against biomechanical insult. Measurement and control of the biomechanics of the trabecular meshwork, ONH, and peripapillary sclera holds the potential for developing a new approach to retinal ganglion cell "neuroprotection" based wholly on targeting the pathophysiological mechanisms underlying IOP regulation dysfunction and IOP-induced axonal damage at the ONH.

IRB Status: None

Disclosures:

J CRAWFORD DOWNS, PHD: No financial relationships to disclose

O092

DEXAMETHASONE MODULATES TRABECULAR MESHWORK CELL MECHANICS

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Purpose: Topical ophthalmic administration of glucocorticoids (GCs), such as dexamethasone (Dex), has been implicated in inducing elevated IOP in a subset of patients with normal IOPs and with individuals with glaucoma. Interestingly, some patients with primary open angle glaucoma (POAG) have increased cortisol levels, an endogenous GC. While GCs have been demonstrated to alter actin within human trabecular meshwork (HTM) cells, changes to the cellular biomechanical attributes have not been investigated.

Methods: Primary human trabecular meshwork cells were isolated from donor corneal buttons. Cells were cultured in the presence or absence of 10^{-7} M Dex for 3 days, following which (a) cell stiffness was determined by atomic force microscopy (AFM), (b) protein was isolated and changes in expression of α SMA, total- and phospho-ERK1/2 were determined by Western blotting, and (c) cytoskeletal structures were visualized by immunocytochemistry and AFM.

Results: Following treatment of Dex, there was an increase in the formation of stress fibers associated with elevated cell stiffness (2.15 ± 0.35 fold), increased expression of pERK1/2 (1.66 ± 0.22 fold) and α SMA (2.31 ± 0.51 fold), with no relative change observed for total ERK expression compared with control cells. Expression of α SMA and pERK1/2 directly correlated with the increase in cellular stiffness.

Conclusions: The dramatic increase in cell stiffness mediated by the activation of ERK and formation of α SMA suggests that Dex increases contractility of HTM cells. Extrapolating the data *in vivo*, we speculate that elevated contractility may have consequences in substantially modifying the extracellular matrix and restricting aqueous outflow.

IRB Status: International

Disclosures:

VIJAY KRISHNA RAGHUNATHAN, PHD: No financial relationships to disclose

O093

ELEVATED PRESSURE INDUCES ECM GENE EXPRESSION CHANGES IN LOW- AND HIGH-FLOW REGIONS OF THE HUMAN TRABECULAR MESHWORK

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Elevated intraocular pressure (IOP) is the primary risk factor for glaucoma. IOP is maintained by changes in aqueous humor outflow resistance, thought to reside within the extracellular matrix (ECM) of the trabecular meshwork (TM). When pressure is elevated, TM cells sense IOP forces as mechanical stretch or ECM distortion and respond appropriately to restore IOP to acceptable levels. This dynamic adjustment process, defined as IOP homeostasis, involves ECM turnover in the TM and is required to maintain outflow resistance. An additional complexity is the segmental nature of aqueous humor outflow around the circumference of the eye, which has implications for resistance adjustments during IOP homeostasis. The objective of this study was to define molecular differences between high and low flow

regions of the TM from human anterior segments perfused at elevated pressure (17.6 mm Hg) for 2 days, in comparison with similar regions perfused at physiological pressure (8.8 mm Hg). ECM and adhesion gene quantitative PCR arrays were performed and fold change gene expression levels were measured. While many ECM genes in both high and low flow regions were down-regulated in response to elevated pressure, some ECM genes were differentially regulated: CTGF, MMP1, MMP3 and SPARC were up in low and down in high flow regions, whereas LAMC1, MMP7, MMP13 and TNC were down in low and up in high flow regions. These gene expression differences give insight into possible molecular mechanisms involved in homeostatic adjustments. Defining molecular differences in the context of segmental outflow is critical to understanding how proper outflow resistance is maintained, and what happens when IOP homeostatic capability is lost, as occurs in glaucoma.

IRB Status: Verified

Disclosures:

JANICE VRANKA, PHD: No financial relationships to disclose

O094

THE ROLE OF BIOMECHANICAL INSULT OF THE OPTIC NERVE HEAD IN THE DEVELOPMENT OF GLAUCOMA

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DEPARTMENT OF OPHTHALMOLOGY AND VISION SCIENCE

Purpose: To investigate the role of astrocyte activation following biomechanical insult.

Methods: Primary rat retinal astrocytes and a rat optic nerve (ON) astrocyte cell line (A7) were stretched (12% @ 1Hz; Flexercell) to induce pathologically relevant biomechanical insult. This was combined with overexpression and knockdown techniques in order to better understand the roles of two proteins, PEA15 and ANXA4, identified from previous proteomic screens in this model. Their function was investigated with regard to apoptosis and the regulation of extracellular matrix remodeling. We investigated the localization and activity of each protein in mouse, rat and human retinæ and in a mouse and pig model.

Results: PEA15 and its targets ERK and Caspase 8 were all up-regulated following biomechanical insult. PEA15 knockdown revealed a two-fold increase in levels of apoptosis compared to control cells. Conversely, transfected PEA15 caused a two-fold decrease in rate of apoptosis. When mechanical insult was combined with PEA15 misexpression, the influence of PEA15 was lost. However, PEA15 regulated levels of MMP2 and MMP9. ANXA4 was localized to the RGC layer and ONH in mouse, rat and human retinas. Live-imaging showed localization to cell and nuclear membranes and decreased membrane dynamics in astrocytes overexpressing ANXA4, suggesting a role in cell viability and membrane stabilization following biomechanical insult. Mechanically induced gliosis in the mouse model exacerbated RGC death >7 fold ($p < 0.01$).

Conclusions: PEA15 is antiapoptotic and regulates secreted

levels of MMPs 2 and 9 in quiescent astrocytes. However, this activity is abrogated when the cells undergo mechanical insult. ANXA4 influences cell viability and membrane stability following insult. These data provide evidence that astrocytes dynamically respond to biomechanical strain, influencing neuronal survival and the extracellular environment in the ONH. Astrocytes are protective when quiescent, but activation increases vulnerability to biomechanical insult.

IRB Status: None

Disclosures:

JOHN FLANAGAN, PHD: Consultant/Advisor relationship with Carl Zeiss Meditec; Grant Support relationship with Carl Zeiss Meditec; Grant Support relationship with Optovue Inc

O095

AGE AND RACE SIGNIFICANTLY IMPACT THE IOP-INDUCED MECHANICAL ENERGY ABSORPTION IN THE POSTERIOR HUMAN SCLERA

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UNIVERSITY OF ALABAMA AT BIRMINGHAM OPHTHALMOLOGY¹;
THE UNIVERSITY OF TEXAS, DEPARTMENT OF BIOSTATISTICS²;
UNIVERSITY OF CALABRIA, MECHANICAL ENGINEERING³

The purpose of this study was to establish if age and race were influential factors in the peripapillary human sclera's ability to absorb mechanical energy from IOP-induced deformation in normal donors of African (AD) and European (ED) descent. Mechanical inflation testing was performed on 28 pairs of normal eyes from human donors (9 AD, 19 ED) aged 20 to 90 years. The intact posterior scleral shell of each eye was pressurized while the full-field, three-dimensional displacements of the outer scleral surface were measured using laser speckle interferometry (ESPI). By analytical differentiation of the displacement field, the mean maximum principal (tensile) strain was computed at 10 discrete pressure levels from 5 to 45 mmHg within the ~2-mm-wide band of peripapillary sclera surrounding the optic nerve head. An asymptotic functional form ($\text{strain} = a + b \cdot \text{IOP} + \text{IOP}/c$) was used to fit the variation of strain with increasing IOP ($R^2=0.99$). The area under the strain-IOP curve, representing the total mechanical energy absorbed by the peripapillary sclera when inflated from 5 to 45 mmHg, was computed by analytical integration. We observed that the ability of the peripapillary sclera to absorb IOP-induced deformation decreased significantly with age in both the AD and ED groups ($p<0.001$), and was more pronounced in the AD group ($p=0.0481$). In conclusion, the peripapillary sclera exhibits a significant loss in its ability to absorb mechanical energy from IOP-induced deformations with age, and the AD eyes showed a significantly more rapid decline in energy absorption with age than ED eyes. These differences may increase the magnitude of transient IOP elevations and thereby contribute to the increased susceptibility of the elderly and persons of AD to glaucoma.

IRB Status: International

Disclosures:

MASSIMO FAZIO, PHD: No financial relationships to disclose

O096

USE OF MICROARCHITECTURAL INFORMATION TO QUANTIFY LAMINA CRIBROSA BIOMECHANICS

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GEORGIA INSTITUTE OF TECHNOLOGY, BIOMEDICAL ENGINEERING

Biomechanical strain acting on glial cells and retinal ganglion cell axons at the level of the lamina cribrosa (LC) has been hypothesized to contribute to glaucomatous optic neuropathy. It is known from the work of Quigley and others that the peripapillary sclera and LC have microarchitectural features expected to significantly affect local biomechanical strain, e.g. a peripapillary scleral fibrous ring and heterogeneous connective tissue density in the LC. Here we combine imaging technologies with computational modeling to quantify such effects. Human and porcine LCs from fresh post mortem eyes were imaged using either 2-photon microscopy or phosphotungstic acid contrast-enhanced micro-computed tomography (μ CT). Lamina cribrosa connective tissue density (volume fraction) and orientation were quantified using image processing techniques, e.g. a Frangi filter for orientation. Peripapillary scleral fiber orientation was measured using small-angle light scattering. Computational models of the posterior globe, including the optic nerve head tissues, were used to evaluate local biomechanical stress and strain in the LC as IOP was changed, and results were compared to simplified models that did not account for LC microstructure. We found that inclusion of μ CT-derived LC microarchitectural information reduced biomechanical tissue strains compared to those computed from a generic model: specifically, first principal strain (a measure of stretch) was 30.8% lower, and maximum shear strain was 30.9% lower. Further, the strain field was more homogeneous when fiber information (orientation) was accounted for. The main driver of local strain variation was LC connective tissue volume fraction. These results are consistent with the idea that LC microarchitecture adapts to reach a target value of local biomechanical strain, a phenomenon similar to that observed in many other connective tissues.

Support: Lawrence Gellerstedt Jr Chair funds, Georgia Research Alliance.

IRB Status: None

Disclosures:

C. ROSS ETHIER, PHD: No financial relationships to disclose

O097

THE IMPLICATIONS OF VARIATION IN THREE-DIMENSIONAL MORPHOMETRY OF THE HUMAN LAMINA CRIBROSA ON THE PATHOGENESIS OF GLAUCOMA

CHRISTOPHER GIRKIN, J. Crawford Downs

UNIVERSITY OF ALABAMA, OPHTHALMOLOGY

The lamina cribrosa provides structural and functional support to the retinal ganglion cell axons as they pass from the relatively high-pressure environment within the eye to the lower pressure region in the retrobulbar cerebrospinal space. To protect the

retinal ganglion cells in this unique anatomic region, the lamina in higher primates has developed into a complex structure composed of a three-dimensional network of flexible beams of connective tissue, nourished by a capillary bed primarily arising from the short posterior ciliary arteries penetrating the immediate peripapillary sclera. Regardless of the primary insult in glaucomatous injury (i.e. vascular or mechanical), IOP-related stress and strain in the laminar connective tissues are key elements in this model and are dependent on the three-dimensional optic nerve head architecture and material properties of these connective tissues. This presentation will summarize our advances in understanding of changes in optic nerve head architecture of the load-bearing connective tissues associated with aging and glaucoma obtained for three-dimensional high-fidelity reconstructions of the load bearing optic nerve connective tissues obtained from normal and glaucomatous donors.

IRB Status: Approved

Disclosures:

CHRISTOPHER GIRKIN, MD, MSPH: No financial relationships to disclose

GL07 – Blood Flow in Glaucoma

O098

REGULATION OF BLOOD FLOW DEPENDENT ON BLOOD PRESSURE AND INTRAOCULAR PRESSURE

LEOPOLD SCHMETTERER

MEDICAL UNIVERSITY OF VIENNA

Regulation of choroidal and optic nerve head blood (CHBF, ONHBF) flow in humans is complex. We set out to investigate the response of CHBF and ONHBF in face of changes in perfusion pressure. In addition, we investigated mechanisms of blood flow regulation focussing on the nitric oxide (NO) system and the endothelin system. All studies were performed in healthy volunteers. Experimental changes in ocular perfusion pressure (OPP) were induced by isometric exercise and an artificial increase in intraocular pressure (IOP). CHBF and ONHBF were assessed using laser Doppler flowmetry. Studies were also done during combined increase in blood pressure and IOP. Studies during changes in OPP were performed in the absence or presence of either a NO synthase (NOS) inhibitor or an endothelin_A receptor antagonist. The choroid and the ONH vasculature showed autoregulatory capacity during both an increase and a decrease in OPP, but some differences between the vascular beds were observed. Administration of a NOS inhibitor significantly decreased CHBF and ONHBF at rest ($p < 0.01$ each), but altered the pressure/flow relationship in the choroid only. By contrast, an endothelin_A-receptor antagonist did not alter basal CHBF or ONHBF, but changed the pressure/flow curve to the right in both vascular beds.

IRB Status: Approved

Disclosures:

LEOPOLD SCHMETTERER, PHD: No financial relationships to disclose

O099

FUNCTIONAL OCT FOR GLAUCOMA

DAVID HUANG

CASEY EYE INSTITUTE – OREGON HEALTH AND SCIENCE UNIVERSITY

Purpose: To measure retinal and optic disc perfusion with optical coherence tomography (OCT).

Methods: A commercial Fourier-domain OCT system (26 kHz RTVue) was used to measure total retinal blood flow (TRBF). A swept-source OCT prototype (100 kHz) was used to produce 3D OCT angiography of optic disc circulation. A disc flow index is then computed.

Results: TRBF was significantly reduced in 42 glaucoma patients, compared to 27 normal subjects. The optic disc flow index was significantly reduced in 11 glaucoma patients, compared to 24 normal subjects. The disc flow index had an inter-visit reproducibility of 4.2% and inter-subject variability of 5.0% among the 24 normal controls. Both TRBF and flow index were highly correlated with visual field parameters in the glaucoma patients, more than structural variables such as nerve fiber layer thickness and optic disc rim area.

Conclusions: Functional OCT may provide useful information for the evaluation of glaucoma that is complementary to structural OCT measurements.

IRB Status: Approved

Disclosures:

DAVID HUANG, MD, PHD: Equity Owner relationship with Optovue; Contracted Research relationship with Optovue; Patents/Royalties relationship with Optovue; Patents/Royalties relationship with Carl Zeiss Meditec

O100

VASCULAR AND CIRCULATORY CHANGES IN GLAUCOMA

CHANDRAKUMAR BALARATNASINGAM, Dao-Yi Yu

LIONS EYE INSTITUTE, PHYSIOLOGY AND PHARMACOLOGY

The importance of circulatory and vascular perturbation in glaucoma pathogenesis has remained a highly debated topic. A large number of histologic, clinical and real-time imaging studies have demonstrated that endothelial and blood flow changes occur in patients with glaucoma, however it remains unclear if these changes are the cause or consequence of glaucomatous optic neuropathy. This presentation will summarize the body of current scientific evidence that speculates upon the role of vascular- and circulation-mediated mechanisms in glaucoma pathogenesis. Findings from key basic science, clinical and imaging studies that have explored this issue will be discussed. The discussion will extend beyond the optic nerve and will also include the findings from investigators that have examined the behavior of the cerebral and extraocular circulations to pressure elevation and glaucoma.

IRB Status: International

Disclosures:

CHANDRAKUMAR BALARATNASINGAM, MD, PHD: No financial relationships to disclose

O101**ENDOTHELIN-1 AND RETINAL VASOMOTOR REGULATION**

TRAVIS HEIN¹, Luke Potts¹, Robert Rosa, Jr.², Lih Kuo¹

TEXAS A&M HEALTH SCIENCE CENTER¹; SCOTT AND WHITE EYE INSTITUTE²

Endothelin-1 (ET-1) is a potent vasoconstrictor involved in physiologic and pathologic regulation of retinal blood flow. Both activation of the ET-1 system and diminished retinal blood flow have been implicated in the pathogenesis of glaucoma. We have recently characterized the cellular and molecular mechanisms contributing to ET-1 system signaling in retinal arterioles, the major regulators of retinal blood flow. To exclude systemic confounding effects, porcine and human (Scott and White IRB-approved study) retinal arterioles (<100 μ m) were isolated for vasomotor and molecular studies. Porcine and human retinal arterioles pressurized to 55 cmH₂O exhibited comparable basal tone and concentration-dependent constriction to ET-1 (0.1 pM to 10 nM). ET-1 precursor big ET-1 also elicited vasoconstriction that was blocked by endothelin-converting enzyme-1 (ECE-1) inhibitor phosphoramidon. ET_A receptor antagonist BQ123 nearly abolished vasoconstrictions to ET-1 and big ET-1. ET_B receptor antagonist BQ788 only slightly reduced responses to ET-1 and big ET-1. ET_B receptor agonist sarafotoxin, in contrast with ET-1, elicited moderate vasoconstriction in a manner sensitive to BQ788 but not BQ123. Endothelial denudation attenuated vasoconstriction to big ET-1 by ~50% without affecting vasoconstrictions to ET-1 and sarafotoxin. Immunohistochemistry revealed ET_A and ET_B receptors predominantly in the smooth muscle and ECE-1 in both endothelium and smooth muscle. Although L-type voltage operated calcium channel inhibitor nifedipine blocked vasoconstriction to protein kinase C (PKC) activator PDBu, vasoconstriction to ET-1 was unaffected. The broad-spectrum PKC inhibitor Gö-6983 abolished vasoconstriction to PDBu, but did not alter ET-1-induced vasoconstriction. Rho kinase (ROCK) inhibitor H-1152 abolished vasoconstriction to ET-1 independent of endothelium. Both ROCK1 and ROCK2 isoforms were expressed in the retinal arteriolar wall. In conclusion, these results provide information on cellular signaling mechanisms involved in retinal arteriolar constriction to ET-1 system activation and may suggest important therapeutic targets for patients with glaucoma related to impaired retinal blood flow.

IRB Status: Approved

Disclosures:

TRAVIS HEIN, PHD: No financial relationships to disclose

O102**OPTIC NERVE HEAD BLOOD FLOW RESPONSE TO INCREASED IOP AND DECREASED BP CHALLENGE IN EXPERIMENTAL GLAUCOMA**

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DEVERS EYE INSTITUTE / LEGACY RESEARCH INSTITUTE¹; DEVERS EYE INSTITUTE²

Increased IOP and decreased blood pressure (BP) are two of the major factors associated with the development of glaucoma. However, studies have shown that lowering BP causes optic nerve head blood flow (ONH BF) and intravitreal oxygen tension decrease, more than that caused by increasing IOP in normal healthy subjects. In our current study, we compared dynamic ONH BF responses to a sudden ocular perfusion pressure (OPP) decrease induced by challenging either BP or IOP in a nonhuman primate experimental glaucoma model with unilateral chronic IOP elevation induced by laser treatment on the trabecular meshwork (N=15). While assessed, the animals were anesthetized with pentobarbital (IV infusion), BP was recorded via direct arterial cannulation and ONH BF (arbitrary unit) was measured by a laser speckle flowgraphy device. The autoregulatory capacity in the ONH was challenged by (1) manometrically increasing the IOP from 10 to 40 mmHg; (2) lowering the BP by releasing pre-inflated cuffs around three limbs, inducing a 16±2mmHg BP drop. The dynamic ONH BF response was recorded 10 seconds before the onset of OPP decrease and continued for one minute. BF response induced by both IOP and BP challenge showed a similar pattern: an initial decrease and a slow recovery. The decrease induced by the IOP or BP challenge took 3.7 and 3.4 seconds to reach the maximal -20% and -16%, respectively; with a falling rate of 0.52%/sec and 0.37%/sec. In pre-laser or contralateral control eyes the response time was significantly delayed (P<0.05). Interestingly, the maximal BF decrease was slightly less (P<0.001) in the IOP induced OPP challenge, but slightly larger in the BP induced OPP decrease (P=0.031). The results suggest that the BP induced a greater OPP decrease and has likely a more detrimental effect on the hemodynamics and subsequently ganglion axons in the ONH.

IRB Status: None

Disclosures:

LIN WANG, PHD: No financial relationships to disclose

O103**CONSIDERATIONS OF VENOUS PRESSURE IN THE PATHOPHYSIOLOGY OF INTRAOCULAR PRESSURE AND OCULAR BLOOD FLOW**

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SALK / PARACELSUS MEDICAL UNIVERSITY, OPHTHALMOLOGY¹; UNIVERSITY OF TEXAS HEALTH SCIENCE CENTER AT SAN ANTONIO, OPHTHALMOLOGY²

The eye has three vascular beds that should be considered in glaucoma, because they influence the dynamics of intraocular pressure and ocular blood flow, the episcleral circulation and the circulations of the optic nerve as well as the uveal circulation. Recent evidence suggests that autonomous innervation from the brain stem modulates episcleral venous pressure. Stimulation of the superior salivatory nucleus results in an elevation of episcleral venous pressure in an acute animal model. Central stimulation also increases uveal blood flow at the same time and the added volume by choroidal engorgement also causes an acute

increase in intraocular pressure. Intraocular pressure on the other hand causes choroidal venous pressure to increase which in turn changes the pressure volume relationship of the eye. The connection between the choroidal venous system and the orbital venous system has long been discussed. Data from our group suggest a passive resistor between these two vascular beds, which means that orbital venous pressure in the upright position of the human body should not feedback to the intraocular venous system. However, this might be different in supine position. Very little is known about the impact of the venous system on the pathogenesis of glaucoma, however, present data suggest, that the venous system might have more impact on our current understanding of the pathophysiology than we are currently taking into consideration for our treatment strategies. The present work gives an overview of the impact of venous pressure on physiological variables and maybe should be considered in the explanation of the pathophysiology of ocular diseases like glaucoma.

IRB Status: Approved

Disclosures:

HERBERT REITSAMER, MD, PHD: Patents/Royalties relationship with AqueSYS; Consultant/Advisor relationship with Novartis; Consultant/Advisor relationship with Aliophta

GL08 – Aqueous Humor Dynamics

O104

MEASUREMENT OF AQUEOUS HUMOR FLOW: PRINCIPLES, PITFALLS, AND PATTERNS

ARTHUR SIT¹, Jay McLaren²

MAYO CLINIC¹; MAYO CLINIC, OPHTHALMOLOGY²

Aqueous humor flow rate can be determined non-invasively by measuring the clearance of a tracer, such as fluorescein, from the anterior chamber and cornea. In this method, fluorescein is instilled in the conjunctival cul-de-sac and a small amount diffuses into the corneal stroma and anterior chamber. Fluorescein leaves the anterior segment primarily through bulk flow, and the aqueous humor flow rate can be determined from the clearance of fluorescein. The concentrations of fluorescein in the anterior chamber and cornea are measured by using a fluorophotometer.

Estimating flow rate from fluorescein clearance requires two assumptions: first that about 90% of fluorescein leaves by bulk flow of aqueous humor and the other 10% leaves by diffusion directly into the blood, and second that the fluorescence accurately represents the mean concentration of fluorescein. If fluorescein leaves the anterior chamber through a path other than bulk flow, such as loss posteriorly through a widely dilated pupil, flow rate can be overestimated by as much as 6 times. If the fluorescein in the anterior chamber or cornea is non-uniform, fluorophotometry could under- or over-estimate the mean fluorescein concentration and flow rate.

This method has been the basis of our understanding of aqueous humor dynamics and has shown flow rates of 2.2 ml/min to 3.1 ml/min during waking hours and a decrease of about 4% per decade of life. It has shown a circadian rhythm with a decrease by

about 50% during sleep. Flow rate does not seem to be affected by transient or chronic changes in IOP, and no form of glaucoma has been associated with a hypersecretion of aqueous humor.

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IRB Status: Approved

Disclosures:

ARTHUR SIT, SM, MD: Consultant/Advisor relationship with AcuMEMS, Inc.; Consultant/Advisor relationship with Allergan, Inc.; Contracted Research relationship with Glaukos, Corp.; Consultant/Advisor relationship with Sensimed, AG

O105

EPISCLERAL VENOUS PRESSURE – MEASUREMENT AND REGULATION

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The episcleral venous pressure (EVP) is the pressure that must be overcome for aqueous humor to exit the eye via the trabecular meshwork pathway, which makes it a principal factor in aqueous dynamics and intraocular pressure (IOP) homeostasis. Indeed, under normal conditions, the EVP is estimated to account for 60 percent of the IOP. However, EVP is difficult to measure, so we know little about it and generally assume it is relatively stable. Given that the episcleral circulation is innervated and that most published EVP measurements were made under local anesthesia, that assumption is probably wrong. Rather, there is growing evidence suggesting EVP is dynamic and regulated. Moreover, EVP is a largely unexplored target for lowering IOP in glaucoma patients, even though it has the potential to lower IOP significantly without the need for intraocular drug penetration. This presentation will provide an overview of the methods used to measure EVP in humans and animal models, and summarize some of the recent studies of EVP regulation.

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IRB Status: Approved

Disclosures:

JEFFREY KIEL, PHD: Contracted Research relationship with Aerie Pharmaceuticals Inc

O106

UVEOLYMPHATIC OUTFLOW PATHWAY

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In most organs, lymphatics maintain tissue-fluid balance by draining extracellular fluid, solutes and proteins. The eye has been considered to be devoid of a lymphatic system; however we reported lymphatic channels in human ciliary body using cell-specific markers and cryo-immunogold electron microscopy. These findings were confirmed in sheep using fluorescent nanoparticles and in the same model, lymphatic drainage from the eye was measured. Using a combination of quantum dots and in vivo hyperspectral imaging, lymphatic drainage from the eye was mapped. Intracamerally injected quantum dots were detected in vivo in the submandibular lymph node. Most recently, latanoprost, a prostaglandin F₂-alpha analog and most widely prescribed antiglaucoma drug, was found to stimulate lymphatic drainage from the eye. Investigating lymphatic drainage using novel in vivo imaging techniques will help to elucidate its role in aqueous humor outflow. Furthermore, development of drugs or biologics that selectively stimulate the uveolymphatic outflow pathway may provide a new strategy of treatment to reduce blindness from glaucoma.

IRB Status: Approved

Disclosures:

YENI YUCEL, MD, PHD: No financial relationships to disclose

O107

THE EFFECTS OF VITRECTOMY AND CATARACT SURGERY ON OXYGEN LEVELS AND DISTRIBUTION IN RHESUS MONKEY EYES

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Glaucoma is a complex, multifactorial disease. Factors such as oxidative stress have been implicated in its development by damaging the cells of the trabecular meshwork (TM), which could lead to increased outflow resistance and elevated intraocular pressure (IOP). The source of reactive oxygen species causing this oxidative damage is unknown. We have described increased molecular oxygen (pO₂) adjacent to the TM in human subjects following pars plana vitrectomy (PPV) and subsequent cataract extraction (CE). The aim of these experiments was to determine whether increased pO₂ in the anterior chamber (AC) after PPV followed by CE affected IOP or outflow facility in monkeys. Five older rhesus monkeys (ages 19-21 years) sequentially underwent PPV and CE plus IOL implantation in one eye and corresponding sham surgeries in the fellow eye. IOP and outflow facility data were obtained at baseline, post-PPV, and post-CE. During surgery, pO₂ measurements were performed (Oxylab pO₂TM, Oxford Optonix) at 4 locations in the AC, the posterior chamber (PC) and vitreous cavity. Aqueous and vitreous humor specimens were obtained following the pO₂ measurements. pO₂ levels increased in the AC angle following vitrectomy (p=0.01) and CE (p=0.001). pO₂ levels were also increased at the lens surface (p=0.001) and in the PC following

CE (p=0.001). IOP increased following PPV and CE, from baseline mean IOP 19.0 mmHg to 22.3 mmHg, (p<0.05). Outflow facility decreased more in the surgery eyes. Further analyses will focus on the antioxidant status of the aqueous and vitreous humor samples. The TM will be examined for structural and cellular changes as well as evidence of oxidative damage. These results suggest an association between increased pO₂ and decreased function of the conventional outflow pathways and are important for understanding the potentially deleterious effects of excess oxygen exposure following common surgical procedures.

IRB Status: Approved

Disclosures:

PAUL KAUFMAN, MD: No financial relationships to disclose

O108

AQUEOUS HUMOR DYNAMIC STRUCTURAL MARKERS OF ONE-YEAR ANGLE SURGERY OUTCOMES

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We seek to identify aqueous humor dynamic (AHD) structural markers associated with one-year clinical outcomes in patients who had angle surgery with intraoperative fluorescein canalograms (IFC). We hypothesize that favorable outcomes are associated with AHD structural markers of the conventional outflow pathway from trabecular meshwork (TM), Schlemm's canal (SC), and downstream scleral venous systems.

Retrospective review of patients who had IFC during canaloplasty or trabeculotomy included demographics, diagnoses, prior lasers and surgeries, medications, glaucoma severity index, intraocular pressure (IOP), surgical complications, blood reflux into the anterior chamber, and post-surgery interventions. High-definition videos (HDV) were recorded during IFC in the presence of a custom cobalt blue filter. HDVs were digitally processed using ImageJ to quantify flow characteristics including retrograde flow from SC through the TM and into the anterior chamber, anterograde flow along and from SC, collector channels, and into episcleral and intrascleral vessels.

There were 16 eyes from 13 patients (62±20.5 yrs; range 15-83 yrs) who met study criteria with diagnoses of primary open-angle, secondary, uveitic, normal tension, or juvenile glaucoma. These eyes had 68 prior procedures averaging 4.3±1.9 including glaucoma, cataract, corneal, or retinal surgeries. Among successful cases (n=12), IOP outcome was 11±2.1 mmHg compared to 17±5.1 mmHg pre-operatively. Medication count dropped from 2.3 pre-operatively to 1.6 at last post-operative visit. Complications included transient hematoma with Descemet's detachment (n=1). Post-surgery interventions included laser goniopuncture (n=5) and laser suture lysis (n=2). IOP outcome and hyphema were not correlated. Preliminary analyses of IFC HDV show variation in flow characteristics.

This case series shows that canaloplasty is possible after prior surgeries; hyphema does *not* correlate with outcome; and preliminary analyses of IFC HDV indicate that flow characteristics through the episcleral and intrascleral veins may be key AHD markers of angle surgery outcomes.

IRB Status: Approved

Disclosures:

SAYOKO MOROI, MD, PHD: Book Royalty relationship with Wolters Kluwer

O109

DO WE NEED A NEW GOLDMANN EQUATION?

CAROL TORIS

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Seventy years ago Hans Goldmann wrote the Goldmann equation describing intraocular pressure (IOP) in terms of the steady state aqueous humor dynamics. Uveoscleral outflow was added to the equation in the 1960s when it was characterized by Anders Bill. The equation includes the terms 1) aqueous production, 2) outflow facility, 3) uveoscleral outflow and 4) episcleral venous pressure. Our understanding of aqueous humor dynamics has advanced over the decades. Therefore, it may be time to consider a new IOP equation.

1) Aqueous production is estimated by determining the flow of aqueous humor through the pupil into the anterior chamber (AF). Some produced fluid moves posteriorly across the vitreous cavity (PF) or moves into the anterior chamber via the iris root (IRF), and neither flow is detected by current measurement techniques. IRF and PF should be added to the equation.

2) Outflow facility is thought to be the hydraulic conductance throughout the trabecular outflow pathway (C_{trab}) but if the trabecular meshwork is removed there remains downstream facility in the pathway (C_d). Additionally, there is a small facility of uveoscleral outflow (C_{fu}) that is usually ignored but is present and can change. C_d and C_{fu} should be added to the equation.

3) Uveoscleral outflow (USO) describes drainage from the anterior chamber angle by a route other than through the trabecular meshwork. Flow through choroidal vessels, lymphatic vessels, sclera, vortex veins and emissarial canals are included in the USO term but if one is interested in only one of these pathways USO should be divided into its component parts.

4) Episcleral venous pressure is the pressure in the veins that drain the aqueous humor from the collector channels. This downstream pressure needs no revision.

This presentation will offer a new IOP equation with the hope that one day all terms will be measurable.

IRB Status: None

Disclosures:

CAROL TORIS, PHD: No financial relationships to disclose

GL09 – IOP-Lowering Agents

O110

CAVEOLAE AND CONVENTIONAL OUTFLOW: PROTEOMIC PROFILING OF OUTFLOW TISSUE CAVEOLAE AND FUNCTIONAL STUDIES IN CAVEOLAE-DEFICIENT MICE

MICHAEL ELLIOTT¹, Stefanie Hauck², Nicole Ashpole³, Xiaowu Gu¹, Mark McClellan¹, Mikhail Dozmorov⁴, Jonathan Wren⁴, Masaki Tanito⁵, Ernst Tamm⁶, Daniel Stamer³

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Gene association studies have linked polymorphisms in the CAV1/2 gene locus to risk of primary open angle glaucoma. These genes encode caveolins, signature proteins of caveolae. The purposes of these studies were to define the caveolae proteome from outflow tissue and determine the consequences of Cav-1 loss on intraocular pressure (IOP), conventional outflow, and outflow pathway morphology. We prepared caveolae from porcine iridocorneal angle tissue by detergent-based and detergent-free methods and carried out quantitative mass spectrometry and bioinformatics. We measured IOP, outflow facility, and outflow tissue morphology/ultrastructure in Cav-1 KO and control mice. Compared to starting membranes, Cav-1 was enriched 5-fold in detergent-free and 35-fold in detergent-based fractions. Mass spectrometry identified 325 and 490 proteins significantly co-enriched with Cav-1 by both methods, respectively. Bioinformatics revealed that more than 50% of canonical pathways significantly overrepresented in caveolae were shared in both preparations. Of these, several pathways relevant to glaucoma including Rho family GTPase signaling, RhoGDI signaling, IL-8 signaling, and Integrin signaling were significantly overrepresented. In Cav-1 KO mice, we found significant and sustained elevations of IOP from 1 to 6 months of age. Pressure-dependent outflow, measured at sequentially increasing pressure steps, was significantly reduced by 43% in Cav-1 KO mice compared to controls. Ultrastructural analysis revealed a loss of caveolae. Schlemm's canal endothelial cells were considerably thicker and shorter than in controls and protruded into the lumen of the canal. Typical giant vacuoles were rare or absent. These results provide compelling evidence that Cav-1 and caveolae play important roles in conventional outflow and IOP regulation plus our proteomic analyses provide a starting point to identify caveolae-dependent mechanisms governing conventional outflow regulation.

IRB Status: None

Disclosures:

MICHAEL ELLIOTT, PHD: No financial relationships to disclose

EFFECT OF INFLAMMATORY CYTOKINES ON AQUEOUS OUTFLOW

TOSHIHIRO INOUE

KUMAMOTO UNIVERSITY

Bioactive molecules, such as growth factors and cytokines, are produced in the eye, regulating the intraocular environment. Of the plethora of ocular diseases, glaucoma may be most affected by aqueous humor bioactive molecules because some elevate IOP by impairing aqueous humor turnover. Recently, we found that the aqueous levels of multiple proinflammatory cytokines, including interleukin 8 and monocyte chemoattractant protein-1 (MCP-1), were elevated in eyes with open-angle glaucoma. In the subsequent study, we clarified that MCP-1 increased aqueous humor outflow facility and decreased transendothelial electrical resistance via CCR2, an MCP-1 receptor, of Schlemm's canal endothelial cells. Interestingly, aqueous MCP-1 levels were elevated in both human and animal eyes after phacoemulsification, and our statistical analysis showed significant relationship in percent changes between concentration of MCP-1 and IOP from baseline levels. These findings indicate that MCP-1 modulates aqueous humor outflow through the conventional pathway. Moreover, the downstream molecules in the intracellular signaling of MCP-1 stimulation might be novel targets for intraocular-pressure lowering therapy in glaucoma patients.

IRB Status: Approved

Disclosures:

TOSHIHIRO INOUE, MD: No financial relationships to disclose

HYDROGEL SUSTAINED-RELEASE OF C3 EXOENZYME LOWERED IOP IN OCULAR HYPERTENSIVE RATS

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To evaluate the IOP-lowering effect of hydrogel sustained-release of C3 exoenzyme in rat eyes. C3 exoenzyme was expressed in *E. coli* and purified by affinity chromatography. Immunofluorescence was performed in NIH 3T3 cells treated with C3 to verify the cellular uptake of the protein. A sustained-release formulation was prepared comprising a bio-absorbable polymer hydrogel and C3 transferase. IOP of Sprague-Dawley rats was measured with the TonoLab rebound tonometer. Baseline IOP was obtained before an intracameral injection of 6 μ l hydrogel (30%) into both eyes to induce an acute ocular hypertension. IOP was measured at 2, 14 and 24h post-injection. After 24h, 6 μ l of the sustained-release formulation (30% hydrogel containing 15 μ g C3 exoenzyme) was injected into the anterior chamber of the right eyes, while 6 μ l hydrogel (30%) was injected into the anterior chamber of left eyes as control. IOP was measured at 2h and then every 12h following the second intracameral injection.

We found that Intracameral injection of hydrogel raised rat IOP to 35–47 mmHg. The sustained-release of C3 exoenzyme significantly lowered IOP. Its maximal IOP reduction effect was 41% (17.2 ± 2.2 mmHg) at 26 h after the second injection ($p < 0.05$). The IOP-lowering effect of C3 exoenzyme lasted about 60h. In conclusion, Hydrogel sustained-release of C3 exoenzyme can reduce IOP in a rat model with ocular hypertension.

IRB Status: None

Disclosures:

XUYANG LIU, MD: No financial relationships to disclose

AUTOTAXIN IS A DRUGGABLE MOLECULAR TARGET TO LOWER IOP AND A POTENTIAL BIOMARKER FOR GLAUCOMA

VASANTH RAO

DUKE UNIVERSITY, DEPARTMENT OF OPHTHALMOLOGY AND PHARMACOLOGY

Primary open-angle glaucoma, the most prevalent form of glaucoma is associated with elevation of intraocular pressure (IOP) resulting from increased resistance to aqueous humor (AH) outflow through the trabecular meshwork (TM) and Schlemm's canal (SC). Although increased resistance to AH outflow in the trabecular pathway is considered to be a main cause for elevated IOP, the specific molecular pathways or factors involved in increased resistance to AH outflow remains to be identified for therapeutic targeting. Our previous studies have demonstrated that the bioactive lipid growth factor- lysophosphatidic acid (LPA), and lysophosphatidylcholine (LPC) generating calcium-independent phospholipase A2 play a significant role in regulation of AH outflow through the TM pathway. LPA perfusion has been demonstrated to increase resistance to AH outflow. The mechanisms regulating levels of extracellular LPA in the AH outflow pathway however, are not understood. Towards this, our recent quantitative proteomics analysis of human AH not only identified autotaxin (ATX) as one of the abundant proteins in AH, and more importantly, confirmed that the LPA producing activity of ATX was found to be significantly elevated in the AH of POAG patients. Autotaxin/LysoPLD, a secretory enzyme which converts LPC to LPA, is the main source of extracellular LPA. Moreover, in our pilot study, topical and intracameral application of a small molecule inhibitor of ATX has been shown to cause a dose-dependent and significant decrease in IOP in normal Dutch-Belted rabbits. Significantly, changes in ATX activity in the AH of live animals can be detected non-invasively by near-infrared imaging using an ATX specific fluorogenic substrate. Based on these different observations, it is reasonable to conclude that ATX is a druggable molecular target to lower IOP and a potential biomarker for glaucoma.

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IRB Status: None

Disclosures:

VASANTH RAO, PHD: No financial relationships to disclose

HIGH THROUGHPUT SCREEN FOR THE IDENTIFICATION OF AGENTS THAT RELAX SCHLEMM'S CANAL ENDOTHELIAL CELLS AND INCREASE OUTFLOW FACILITY

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Aqueous outflow through the conventional pathway has been intimately linked to the contractile state of Schlemm's canal (SC) endothelial cells (Zhou et al. 2011, *Journal of the Royal Society, Interface*. DOI: 10.1098). Recently, we developed a high throughput platform to measure traction forces of SC cell monolayers and applied this new technology to discover drugs that relax SC, but not trabecular meshwork (TM) cells. Human SC and TM cells were isolated from human donor eyes as previously described (Stamer et al. 1995, 1998, *IOVS*). Cell monolayers were cultured on collagen I-coated polyacrylamide gels and labeled with fluorescent beads. Traction forces were obtained using image analysis and Fourier transform traction calculation based on bead displacements. Using a 96-well plate format, we screened the Prestwick library of 1200 FDA-approved drugs for those that significantly relaxed SC, but not TM cells. Positive controls included isoproterenol and Y27632, which are both known to relax SC cells. We perfused candidate compounds into enucleated mouse eyes as a secondary screen, examining effects on outflow facility. Results from the screen identified 17 drugs that relaxed SC cells including nine β 2-adrenergic receptor agonists, which were expected to relax SC cells. The most potent agent of the 17 was the prostaglandin compound, alprostadil, having no effect on TM cells but significantly reduced SC cell contractility by up to 80% in a dose-dependent manner. Importantly, alprostadil (1 μ M) was tested and found to increase outflow facility in enucleated mouse eyes (0.028 ± 0.006 versus 0.037 ± 0.01 μ l/min/mmHg, $n=11$ pairs, $p=0.029$). Taken together, data show that a high throughput assay based on a physiological endpoint, cell relaxation, effectively identifies candidate glaucoma drugs. The most efficacious drug, alprostadil, significantly increased outflow facility in mouse eye demonstrating the utility of the screen for identifying compounds that lower intraocular pressure.

IRB Status: None

Disclosures:

DANIEL STAMER, PHD: No financial relationships to disclose

SOLUBLE GUANYLATE CYCLASE: AN EMERGING THERAPEUTIC TARGET IN PRIMARY OPEN-ANGLE GLAUCOMA

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Glaucoma is a progressive optic neuropathy characterized by visual field defects that ultimately lead to irreversible blindness. By the year 2020, an estimated 80 million people will have glaucoma, 11 million of which will be bilaterally blind. Primary open-angle glaucoma (POAG) is the most common type of glaucoma. Elevated intraocular pressure (IOP) is currently the only risk factor amenable to treatment of POAG. However, in many cases, available therapies offer incomplete protection, and POAG often goes undetected until irreparable damage has been done, highlighting the need for novel therapeutic approaches, drug targets, and biomarkers. In addition, the molecular signaling involved in the pathogenesis of POAG remains unknown. In this presentation, the role of the nitric oxide (NO) receptor soluble guanylate cyclase (sGC) that generates the secondary signaling molecule cyclic guanosine monophosphate (cGMP) in the regulation of IOP and in the pathophysiology of POAG will be discussed. Mice lacking the alpha1 subunit of sGC represent a novel and translatable animal model of POAG, characterized by thinning of the retinal nerve fiber layer and loss of optic nerve axons in the context of an open iridocorneal angle. The optic neuropathy associated with soluble guanylate cyclase sGC alpha1-deficiency is accompanied by modestly increased intraocular pressure. Moreover, data from a candidate gene association study suggests that a variant in the locus containing the genes encoding for the alpha1 and beta1 subunits of soluble guanylate cyclase is associated with POAG in patients presenting with paracentral vision loss, a disease subtype thought to be associated with vascular dysregulation. These findings provide new insights into the pathogenesis and genetics of POAG and suggest new therapeutic strategies for POAG.

IRB Status: Approved

Disclosures:

EMMANUEL BUYS, PHD: No financial relationships to disclose

GL10 – Hot Topics in Glaucoma

GENETIC AND PHARMACOLOGICAL REDUCTION OF ER STRESS IN THE TRABECULAR MESHWORK RESCUES GLAUCOMA IN MOUSE MODELS OF GLAUCOMA

GULAB ZODE

NORTH TEXAS EYE RESEARCH INSTITUTE

Primary open angle glaucoma (POAG) is the most common form of glaucoma and is accompanied by elevated intraocular pressure (IOP) resulting from increased resistance to aqueous humor outflow through the trabecular meshwork (TM). Pathological mechanisms underlying increased outflow resistance have not been delineated. Recently, we have developed a mouse model of glucocorticoid-induced glaucoma and a transgenic mouse model that expresses human mutant myocilin and develops adult onset primary open angle glaucoma (*Tg-MYO-C^{Y437H}* mice). Here, we examined whether increased endoplasmic reticulum (ER) stress in the TM is associated with elevation of intraocular pressure (IOP) and further determined whether

reducing ER stress rescues glaucoma in these mouse models of glaucoma. TM tissues obtained from these mouse models were utilized to examine ER stress markers by Western blot, immunostaining and RT-PCR analysis. We demonstrate that ocular hypertension induced by topical ocular 0.1% dexamethasone (DEX) or by mutant human myocilin is associated with chronic ER stress in the TM. Both dexamethasone and mutant myocilin activated the unfolded protein response including phosphorylation of IRE-1 α and eIF-2 α , increase levels of GRP78, GRP94, and ATF-4, and also induce pro-apoptotic transcriptional factor Chop in the TM (n=4 in each group; t-test, $P<0.001$). Deletion of Chop protected IOP elevation in *Tg-MYOC^{Y437H}* mice and DEX-induced ocular hypertension by reducing ER stress in the TM. Furthermore, the chemical chaperone, sodium 4-phenylbutyrate (PBA) rescued DEX-induced ocular hypertension and prevented glaucoma in *Tg-MYOC^{Y437H}* mice by reducing ER stress in the TM. Our studies indicate that ER stress plays a critical role in ocular hypertension in glaucoma, and the reduction of ER stress via PBA can be used as a therapeutic strategy for the treatment of glaucoma.

IRB Status: International

Disclosures:

GULAB ZODE, PHD: Contracted Research relationship with Novartis Inc.

O117

EPIGENETIC REGULATION OF GLAUCOMA-ASSOCIATED FACTORS IN THE TRABECULAR MESHWORK

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UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER

Purpose: A number of glaucoma-associated growth factors contribute to the pathological changes in the trabecular meshwork (TM) that lead to elevated intraocular pressure (IOP) in primary open angle glaucoma (POAG). Abnormal levels of growth factors have been reported in the aqueous humor and/or TM cells/tissues from POAG patients. However, the etiology of the dysregulation of these growth factors is still not clear. We hypothesize that epigenetic mechanisms may play a role in this dysregulation and contribute to IOP elevation as well as POAG.

Methods: Primary human TM (HTM) cell cultures were treated with or without the histone deacetylase inhibitor (HDACi) Thapsigargin (TDP-A) at 10nM for 4 days. Cells were harvested and RNA was isolated for qPCR. Bovine anterior segments were dissected from fresh eyes for perfusion organ culture (POC) for 7-10 days. One of the paired eyes was treated with TDP-A while the fellow eye was treated with 1% DMSO as a control. IOP was monitored during experiments and perfusates were collected for Western immunoblotting (WB).

Results: TDP-A increased the expression of TGF β 2 and sFRP1 by about 4 fold (N=3, $p<0.01$). It also elevated IOP in POC eyes. WB showed that the level of TGF β 2 and sFRP1 increased in perfusates collected from TDP-A treated eyes.

Conclusion: We found that TDP-A is a potent HDACi that is able to increase the expression POAG-associated growth

factors TGF β 2 and sFRP1 in both TM cells and tissues. It also elevates IOP in perfusion cultured bovine eyes. Further studies are required to determine whether this IOP elevation mediated by HDACi results from increasing the expression of POAG-associated growth factors, and what types of histone deacetylase are involved.

IRB Status: None

Disclosures:

WEIMING MAO, PHD: No financial relationships to disclose

O118

GLAUCOMA: AN INSIGHT INTO EPIGENETIC REPROGRAMMING

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Glaucoma is a chronic progressive optic neuropathy and the second most common form of irreversible blindness and is thought to affect approximately 60 million people worldwide. Current treatment for glaucoma focuses on lowering intraocular pressure (IOP), but despite well-controlled IOP some patients continue to suffer progressive damage. Other possible methods of treatment include targeting the fibrotic pathology associated with glaucoma. In glaucoma fibrosis is observed in the lamina cribrosa (LC) and trabecular meshwork (TM) regions, with increased expression of pro-fibrotic genes such as transforming growth factor beta (TGF β). These areas are also subjected to a hypoxic environment in primary open angle glaucoma (POAG). Hypoxia has been shown to induce epigenetic changes in many fibrotic diseases. The purpose of this study was to investigate the role of hypoxia-driven changes in epigenetic regulation of TGF β in primary human LC and TM cells from donors with (G) and without (N) POAG. An increased level of global DNA methylation (5-MeC) as determined by ELISA was observed in GLC cells compared to NLC cells ($P<0.05$). A similar finding was found in NTM compared with GTM cells. qPCR analysis showed increased mRNA expression of DNA methyltransferases (DNMT) and TGF β 1 in GLC cells compared to NLC cells ($P<0.05$). Methylation specific PCR analysis of the TGF β promoter illustrated increased levels of unmethylated DNA in GLC cells. Hypoxia (1%O₂) increased the level of global DNA methylation in NTM cells compared to normoxic cells (21%O₂) ($P=0.3$), along with increased TGF β 1 expression ($P<0.01$). In conclusion we have shown that the hypoxic environment in glaucoma can affect epigenetic mechanisms specifically methylation. Furthermore, expression of key fibrotic genes such as TGF β is also affected. Available epigenetic modulators may offer an attractive therapeutic approach to alter the fibrotic phenotype associated with glaucoma.

IRB Status: International

Disclosures:

DEBORAH WALLACE, PHD: No financial relationships to disclose

GENETIC ANALYSIS OF SUSCEPTIBILITY TO RETINAL GANGLION CELL LOSS USING INBRED MICE: THE STORY OF SPINK2

ROBERT NICKELLS, Cassandra Schlamp

UNIVERSITY OF WISCONSIN

Glaucoma is a complex genetic disease, likely influenced by the interaction of multiple different loci. Quantitative trait analysis using inbred mice may provide an important tool to identify chromosomal regions, genes, and/or biochemical pathways that influence ganglion cell soma or axon loss, and thus may factor into the susceptibility, rate of progression, and severity of glaucoma in humans. Using controlled optic nerve crush on 15 different inbred strains of mice, we identified a region of mouse chromosome 5 that confers dominant resistance to neuronal death. This locus was designated Retinal ganglion cell susceptible 1 (*Rgcs1*). Resistant mice (DBA/2J) congenic for the susceptible *Rgcs1* allele (from BALB/cByJ mice) exhibit greater susceptibility to acute optic nerve crush and a more severe glaucoma phenotype. Further genetic and molecular characterization of the *Rgcs1* allele has identified the gene Serine protease inhibitor Kazal type 2 (*Spink2*) as the likely candidate underlying the cell death phenotype. Expression of this gene is generally localized in cells of the ganglion cell layer, and dying cells appear to dramatically up-regulate protein levels. Susceptible mice express higher levels of a variant (T19S) of *Spink2*. Cell culture studies indicate that cells expressing an exogenous *Spink2* construct are more susceptible to an apoptotic stimulus, with the BALB/cByJ variant conferring the greatest susceptibility. How *Spink2* affects cell susceptibility is not known. Cells overexpressing SPINK2 protein exhibit more rapid aggregation of BAX at mitochondrial surfaces and an increase in the accumulation of LC3-II containing autophagic vesicles. We propose that SPINK2 lowers the threshold for a cell to convert from a protective autophagic mechanism to the activation of molecular pathways associated with apoptosis.

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IRB Status: None

Disclosures:

ROBERT NICKELLS, PHD: No financial relationships to disclose

EXCITOTOXIC RGC DEATH DOES NOT REQUIRE JUN OR TNF

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FLAUM EYE INSTITUTE, UNIVERSITY OF ROCHESTER

Excitotoxicity has been suggested to contribute to retinal ganglion cell (RGC) death in many diseases. To understand the molecular mechanisms involved in RGC death after an excitotoxic injury, we first characterized the pattern of cell death after intravitreal NMDA injection (2ul of 20 or 80mM). Seven days after NMDA injections there was a ~70% loss of RGCs (TUJ1+ cells) and a clear loss of amacrine cells (AP2a+ cells). The transcription factor JUN is critical for RGC death after a variety of insults and is upregulated in RGCs after an excitotoxic insult. In addition to a potential cell-intrinsic role in RGCs, JUN-dependent RGC-extrinsic pathways may also contribute to excitotoxicity-induced RGC death. A recent study suggested that the cytokine TNF released by Müller glial cells is required for RGC death after NMDA insult, at least at early stages after insult (6 hours after insult; Lebrun-Julien et al., J. Neurosci, 2009). Interestingly, JUN can control injury induced TNF expression. After intravitreal injection of NMDA, JUN was expressed in Müller glial cells, RGCs and amacrine cells. Despite JUN's upregulated expression in retinal cells after NMDA insult, *Jun* deficiency (floxed allele recombined with Six3-cre) did not prevent RGC death (given as % of control PBS injection; *Jun*^{+/+}, 27.4 ± 3.7%; *Jun*^{-/-}, 28.8 ± 3.1%; P = 0.499). Furthermore, despite the protection provided by *Tnf* deficiency at early time points after NMDA insult (Lebrun-Julien et al., J. Neurosci, 2009), *Tnf* deficiency did not provide protection from excitotoxic insult at 7 days (given as % of control PBS injection; *Tnf*^{+/+}, 21.9 ± 1.2%; *Tnf*^{-/-}, 31.7 ± 2.0%; P = 0.108). Thus neither TNF nor JUN appears to be required for RGC death. In the future, it will be important to determine the molecular mechanisms killing not only RGCs, but also amacrine cells.

IRB Status: None

Disclosures:

KIMBERLY FERNANDES, PHD: No financial relationships to disclose

Cornea and Ocular Surface

CO01 – The Role of Mucins in Ocular Surface Barrier Function

O121

COMPARISON OF THE TRANSMEMBRANE MUCINS MUC1 AND MUC16 IN HUMAN CORNEAL EPITHELIAL BARRIER FUNCTION

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Membrane-anchored mucins are present in the apical surface glycocalyx of mucosal epithelial cells, each mucosal epithelium having at least two of the mucins. The mucins have been ascribed barrier functions, but direct comparisons of their functions within the same epithelium have not been done. In a human corneal epithelial cell line that expresses the same membrane anchored mucins present in native epithelia, MUC1 and MUC16, the mucins were independently and stably knocked down using shRNA. Barrier functions tested included dye penetrance, bacterial adherence and invasion, transepithelial resistance, tight junction formation, and apical surface size. Knockdown of MUC16 decreased all barrier functions tested, causing increased dye penetrance and bacterial invasion, decreased transepithelial resistance, surprisingly, disruption of tight junctions, and greater apical surface cell area. Knockdown of MUC1 did not decrease barrier function, in fact, barrier to dye penetrance and bacterial invasion increased significantly. These data suggest that barrier functions of membrane-anchored mucins vary in the context of other membrane mucins, and MUC16 provides a major barrier when present.

IRB Status: Verified

Disclosures:

ILENE GIPSON, PHD: No financial relationships to disclose

O122

GALECTIN-MUCIN INTERACTIONS IN OCULAR SURFACE HEALTH AND DISEASE

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Carbohydrates have been traditionally considered sources of energy for the living organism. However, during the last few years, it has become evident that carbohydrates also play important roles in determining cell function. A large amount of carbohydrates are located on the cell surface, where they modulate a wide variety of biological processes, such as host-pathogen interactions, cell communication, and immune response. Recent progress in the field of glycobiology is being facilitated by the development of new methodologies for the high-through-

put analysis of glycans—known as glycomics. Our laboratory has been using these methodologies to study the glycogenes expressed at the ocular surface in normal and pathological conditions. Mucins and galectins are among the most highly expressed glycogenes on the surface of the eye, where they contribute to maintain homeostasis and act as a selective barrier to molecules and pathogens. Understanding the mechanisms by which glycoconjugates contribute to the protection of epithelial surfaces could help with the development of new therapeutic approaches for patients with ocular surface disease.

IRB Status: None

Disclosures:

PABLO ARGÜESO, PHD: No financial relationships to disclose

O123

CLUSTERIN (CLU) PREVENTS OCULAR SURFACE DAMAGE IN A MOUSE MODEL FOR HUMAN DRY EYE DISEASE

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UNIVERSITY OF SOUTHERN CALIFORNIA, INSTITUTE FOR GENETIC MEDICINE

MMP9 is a key mediator of ocular surface damage due to dry eye. Clusterin (CLU) is an extracellular chaperone protein, expressed at high levels in corneal epithelium. We recently reported that CLU inhibits the proteolytic activity of MMP9 and MMP9-mediated damage to epithelial barrier function and that expression of CLU in the corneal epithelium is greatly reduced under dry eye conditions. We hypothesized that supplementation of CLU levels would protect the ocular surface against the damaging effects of dry eye. Desiccating stress was created at the ocular surface of female C57BL/6J mice using the air draft plus scopolamine protocol. At the same time, 1 ul of recombinant human sCLU (secreted form of CLU) formulated at 0.1- 10 ug/ml in PBS, was applied topically to the ocular surface 4 times daily for 5 days. Corneal epithelial barrier function was quantified by measuring permeability to carboxyfluorescein (CBF) dye. We also tested CLU effect on TNF-alpha-induced MMP-9 expression. CBF uptake in eyes stressed for 5 days while also being treated with PBS alone was 10-fold greater than baseline. In contrast, CBF uptake in eyes stressed for 5 days while also being treated with sCLU was only 1.7-fold (1 ug/ml) or 1.4-fold (10 ug/ml) greater than baseline. Treatment of 0.1 ug/ml sCLU displayed no protective effect, showing the uptake level similar to the PBS control, however, when desiccating stress was relieved by stopping scopolamine injection, it facilitated the recovery to the base line uptake twice faster than PBS control. Treatment of corneal epithelial cells *in vitro* with sCLU significantly reduced the stimulatory effects of TNF-alpha on MMP-9 expression. CLU protects the ocular surface against the damaging effects of desiccating stress in mice, which models human dry eye disease. It may also promote the recovery of damaged ocular surface.

IRB Status: Approved

Disclosures:

SHINWU JEONG, PHD: No financial relationships to disclose

O124

ROLE OF THE MEMBRANE-ANCHORED MUCINS MUC1 AND MUC16 IN SUPPRESSING TOLL-LIKE RECEPTOR-MEDIATED INNATE IMMUNE RESPONSES AT THE OCULAR SURFACE

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SCHEPENS EYE RESEARCH INSTITUTE, MASSACHUSETTS EYE AND EAR¹; SERI-MEE, HARVARD MEDICAL SCHOOL²

Toll-like receptor (TLR)-mediated innate immune responses mounted against invading pathogens are typically considered to be beneficial to the host. Paradoxically, these responses, if left unchecked, can lead to unwarranted inflammation and cause bystander tissue damage. Therapeutically, there is growing interest in studying mechanisms or identifying factors that regulate such responses. This project was undertaken to test the hypothesis that the membrane-associated mucins (MAMs), MUC1 and MUC16, expressed on the apical surface of the corneal epithelium, are involved in suppressing TLR-mediated innate immune responses by limiting interactions between TLRs and their corresponding ligands. Indeed, results from our experiments revealed that differentiated cultured corneal epithelial cells knocked down for the MAMs MUC1 and MUC16, upon exposure to the TLR2 and TLR5 agonists, exhibited increased message levels of the pro-inflammatory cytokines and chemokine IL-6, TNF- α , and IL-8, in comparison to non-transfected and control-transfected cells. Similarly, IL-6 and TNF- α message levels were increased in the corneal epithelium of Muc1^{-/-} null and Muc1^{-/-}Muc4^{-/-}Muc16^{-/-} null mice, in comparison to wild type mice, following exposure of enucleated eyes to the TLR2 and TLR5 agonists. Overall, our results suggest that the MAMs MUC1 and MUC16, by suppressing TLR-mediated innate immune responses, contribute to the maintenance of immune homeostasis at the ocular surface.

IRB Status: None

Disclosures:

BALARAJ MENON, PHD: No financial relationships to disclose

O125

ALTERED MUCIN GLYCOSYLATION IN THE PATHOGENESIS OF AQUEOUS-DEFICIENT DRY EYE

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UNIVERSITY OF CALIFORNIA, SAN FRANCISCO, FRANCIS I. PROCTOR FOUNDATION

In autoimmune diseases like ocular cicatricial pemphigoid, Stevens-Johnson syndrome and Sjögren's syndrome, severe aqueous tear deficiency provokes squamous metaplasia (SQM),

a devastating, end-stage consequence that represents a multi-step, immune-mediated process resulting in pathological keratinization of the cornea and altered goblet and non-goblet cell (GC) mucins. Mucins are integral components of the mucosal epithelium and tear film that play essential roles in maintaining ocular surface integrity and tear film stability. Modification of secreted mucins can provoke changes in lubrication and protection of the mucosal surface. We established mice deficient in the autoimmune regulator (Aire) gene as a model for autoimmune-mediated, aqueous-deficient dry eye disease and SQM. In Aire-deficient mice, CD4⁺ T cells represent the main effector cells and local signaling via the IL-1/IL-1R1 pathway provides an essential link between autoreactive CD4⁺ T cells and ocular surface disease. Moreover, Aire KO mice experience a robust acidification and gradual loss of GC mucins that is reversed when they lack functional IL-1R1. Using histochemical stains and sialic acid-specific lectins, we discovered that the link between IL-1R1-mediated ocular inflammation and GC mucin acidification occurred as the result of increased sialylation of terminal galactose residues via galactosyl (α -2,3) bonds. Significantly, fewer MAL1-positive GCs were noted in the conjunctiva when eyes of Aire KO mice were treated with the IL-1R1 antagonist, Anakinra, with a corresponding decrease in the expression of pathological keratinization marker, small proline-rich protein-1B. Further analysis by Glyco-Gene Chip microarray confirmed significant upregulation of IL-1 alpha and beta cytokines in Aire KO mice and identified several differentially expressed glycosyltransferases potentially responsible for altered ocular surface glycosylation. Thus, targeting the IL-1/IL-1R1 signaling pathway through topical administration of IL-1RA may provide a novel option to improve ocular surface integrity, increase tear secretion and restore the normal glycosylation pattern of GC mucins in the setting of chronic inflammation.

IRB Status: Verified

Disclosures:

DENISE STEPHENS, PHD: No financial relationships to disclose

CO02 – Immune Regulation of Cornea and Anterior Segment

O126

DYSREGULATION OF TREGS IN CORNEAL INFLAMMATION

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SCHEPENS EYE RESEARCH INSTITUTE, MASSACHUSETTS EYE AND EAR, DEPARTMENT OF OPHTHALMOLOGY, HARVARD MEDICAL SCHOOL

The mouse model of dry eye disease (DED) offers a useful model for the study of autoimmune disease: the pathophysiology is driven principally by CD4⁺ IL-17-secreting T helper-17 (Th17) cells; there is clear development of immunologic memory; and the disease can be induced in other (healthy) animals by adoptive transfer of specific T cell subsets. Furthermore, the model has been useful for answering two additional questions: First, what is the defect in T regulatory (Treg) function that facili-

tates/augments disease severity in DED? Second, how does autoimmunity affect the generation of alloimmunity? In regard to the first question, myriad lines of research from our lab now indicate that there is a significant defect in Treg function in DED: these Tregs are less able to suppress T cell, and in particular Th17, proliferation. This defect appears to be correlated with high expression levels of specific Th17-associated cytokines such as IL-6 and IL-23. Second, our data definitively demonstrate that DED-related autoimmunity significantly enhances generation of alloimmunity in a mouse model of corneal transplantation. This brief presentation will provide an overview of T cell-mediated immune mechanisms in DED, and also highlight the failure of T regulatory cells in controlling ocular surface inflammation in this autoimmune condition of the ocular surface.

IRB Status: Approved

Disclosures:

REZA DANA, MD, MPH, MSC: Consultant/Advisor relationship with Alcon; Consultant/Advisor relationship with Allergan; Consultant/Advisor relationship with Bausch & Lomb; Investor relationship with Eleven Biotherapeutics; Consultant/Advisor relationship with Genentech

O127

THE TWO FACES OF INTERFERON- γ IN CORNEAL TRANSPLANT SURVIVAL AND REJECTION

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UNIVERSITY OF TEXAS, SOUTHWESTERN MEDICAL SCHOOL, OPHTHALMOLOGY

Previous dogma posited that interferon-gamma (IFN- γ)-producing CD4⁺ Th1 cells were the primary mediators of corneal allograft rejection. However, IFN- γ knockout (KO) mice reject 100% of corneal allografts disparate with the recipient at the major histocompatibility complex (MHC) and all known minor histocompatibility (minor H) gene loci (= fully allogeneic). We examined the effect of IFN- γ depletion on MHC-matched, minor H-mismatched corneal allografts (NZB donors \rightarrow BALB/c recipients). Experiments also examined the effect of IFN- γ depletion on graft survival in mice with allergic conjunctivitis, which is a risk factor for corneal allograft rejection. We evaluated the effect of IFN- γ on allograft survival and the generation of CD4⁺CD25⁺ T regulatory cells (Tregs). Fully allogeneic corneal allografts were rejected in 50% of mice with an intact IFN- γ gene. By contrast, IFN- γ KO mice or WT mice treated with anti-IFN- γ neutralizing antibody failed to develop Tregs and rejected 100% and 90% of their fully allogeneic corneal allografts respectively. The opposite occurred in mice that received minor H-mismatched grafts; WT mice rejected 80% of their minor H-mismatched grafts while 90% to 100% of these grafts survived in IFN- γ deficient hosts. Importantly, IFN- γ depletion resulted in the emergence of donor-specific Tregs in minor H-disparate graft recipients. 100% of minor H-mismatched grafts transplanted to mice with allergic conjunctivitis underwent rejection, while administration of anti-IFN- γ antibody abolished the high-risk condition and resulted in 80% graft survival. These results indicate that IFN- γ can either promote or prevent corneal allograft rejection depending on

the array of histocompatibility antigens that confront the host. In the United States, MHC matching is normally not employed. However, our findings suggest that MHC matching combined with neutralizing IFN- γ can convert the high risk host to a normal risk host and promote corneal allograft survival.

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IRB Status: Approved

Disclosures:

JERRY NIEDERKORN, PHD: Consultant/Advisor relationship with Allergan

O128

IS CONJUNCTIVA-ASSOCIATED LYMPHOID TISSUE INVOLVED IN OCULAR SURFACE IMMUNE REGULATION?

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UNIVERSITY OF COLOGNE, DEPARTMENT OF OPHTHALMOLOGY¹; ALLERGAN INC., BIOLOGICAL SCIENCES²; UNIVERSITY OF TEXAS, SOUTHWESTERN MEDICAL SCHOOL, OPHTHALMOLOGY³

Introduction: Conjunctiva-associated lymphoid tissue (CALT) is generally present at the ocular surface demonstrating alterations in inflammatory diseases such as chlamydia infection, adenoviral infection, ocular allergy, dry eye and toxic conjunctivitis. Although antigen-uptake, presentation and induction of a secretory immune response is likely to take place in CALT, until now, only limited information is available on a possible immunoregulatory role.

Materials/Methods: Using different BALB/c and C57BL/6 mice and by challenging the ocular surface by repeated topical antigen-stimulation with OVA and KLH and by inducing short-ragweed (SRW) ocular allergy as well as dry-eye disease a broad spectrum of conjunctival immune responses (Th1 vs. Th2) were induced. In each model CALT was investigated by means of immunohistochemistry to characterize cellular composition and overall expression rate. In addition intravital microscopy was applied to analyze cellular migration.

Results: CALT is present under different pathological conditions at the ocular surface of mice, mimicking the human situation in most terms. Whereas CALT expression is significantly increased following OVA, KLH and SRW challenge, in dry eye disease and particular are in C57BL/6 mice CALT follicles are strongly reduced. CALT frequently contains (CD25⁺FOXP3⁺CD4⁺) regulatory T-cells besides CD4⁺ and CD8⁺ T-cells, B-cells and antigen-presenting cells, that demonstrate spatial organization similar to lymphnodes and spleen. Cellular migration takes place within the lymphoid follicle and the adjacent lymphoepithelium as well as rapid migration within different mucosal compartments.

Discussion: Based on the findings that CALT is significantly reduced in experimental dry eye disease, a concurrent reduction of CALT and containing Tregs may foster the T-cell driven autoimmune response. To investigate the role of CALT in regulating the immune response e.g. via Tregs, experiments are currently

conducted that use mice with strongly increased CALT in order to prevent or reduce autoimmune responses in experimental dry eye.

IRB Status: Approved

Disclosures:

PHILIPP STEVEN, MD: No financial relationships to disclose

O129

REGULATION OF OCULAR INFLAMMATION BY MESENCHYMAL STEM CELLS

SUNIL CHAUHAN

SCHEPENS EYE RESEARCH INSTITUTE / MASSACHUSETTS EYE AND EAR, DEPARTMENT OF OPHTHALMOLOGY, HARVARD MEDICAL SCHOOL

Along with their capacity for differentiating into cells of multiple lineages, mesenchymal stem cells (MSC) have generated great interest due to their unique anti-inflammatory and immunomodulatory properties. While the ability of MSC to suppress pathogenic immune cell function has been well defined, little is known regarding the mechanisms by which MSC promote the immunomodulatory function of regulatory immune cells, such as regulatory T cells (Tregs). Tregs are critical in modulating immunity in a variety of ocular inflammatory diseases, including autoimmune uveoretinitis, dry eye disease, corneal transplant rejection and allergic conjunctivitis. In the present study, using a mouse model of corneal transplantation, we delineated the critical mechanisms by which MSC directly promote Treg function in order to reestablish ocular immune quiescence and promote allograft survival.

IRB Status: Approved

Disclosures:

SUNIL CHAUHAN, DVM, PHD: No financial relationships to disclose

O130

OCULAR NEUROPEPTIDE REGULATION OF INNATE IMMUNITY AND ANTIGEN PRESENTATION

ANDREW TAYLOR

BOSTON UNIVERSITY SCHOOL OF MEDICINE

The healthy ocular microenvironment suppresses inflammation, and induces immune regulation. This is mediated partly by neuropeptides alpha-melanocyte stimulating hormone (α -MSH) and Neuropeptide Y (NPY). Together α -MSH and NPY induce myeloid suppressor cell characteristics in macrophages. In addition, these macrophages while not suppressed in Fc-receptor (FcR) mediated phagocytosis of opsonized bioparticles, do appear to be suppressed in activating phagolysosome acidification. Therefore, the α -MSH/NPY treated phagocytes were assayed to see whether activation markers mediated by FcR-phagocytosis are suppressed, whether the suppression of phagolysosome acidification is from suppressed phagolysosome

some formation, and whether there is suppression of antigen processing. Mouse monocytes were treated with α -MSH and NPY and fed antibody-opsonized E. coli bioparticles. The cells were analyzed after incubation by flow cytometry for H-2K^d, Ia^d, CD40, CD80, and CD86 expression. Other monocytes were treated, but fed magnetic beads coated with ovalbumin and opsonized anti-ovalbumin IgG. The cells after incubation were lysed and intracellular vesicles containing the beads were isolated by magnetic sorting. The isolated vesicles were assayed by immunoblotting for Rab5, Rab7, and ovalbumin. Phagocytosis enhanced expression of all the activation markers except the constitutive expression of H-2K^d was unchanged. There was a significant suppression of CD40 and CD80 expression with no change in Ia^d and CD86 by the α -MSH/NPY treated phagocytes. The immunoblotting showed that the levels of Rab7 to Rab5 were significantly suppressed in the treated phagocytes, indicating that there was suppression of phagolysosome formation. In addition, the phagosomes contained 2-3 times more intact ovalbumin protein. Therefore, along with α -MSH and NPY inducing anti-inflammatory cytokine production, they divert the processing of phagocytized materials, and any antigen presentation will be without the full range of co-signals. This activity may be one of the mechanisms through which the ocular microenvironment can minimize presentation of auto-antigens, and activation of autoreactive effector T cells.

IRB Status: None

Disclosures:

ANDREW TAYLOR, PHD: No financial relationships to disclose

O131

REGULATORY PMN CONTROL T CELL RESPONSE SEX-SPECIFICALLY THROUGH LIPOXIN A4-MEDIATED PROCESS

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Amplified T cell response is a pivotal event in sex-specific pathogenesis of Dry Eye Disease (DED). A protective and immune-regulatory lipid mediator circuit (lipoxin) is highly expressed in ocular surface and markedly amplified by PMN. This lipid circuit is down regulated by estrogen and drives sex-specific differences in corneal wound healing. If PMN-lipoxin circuit has a role in the pathogenesis of DED and lymphocyte activation is unknown. We investigated sex-specific difference and a potential role of PMN and lipoxin circuit in regulating immune responses in the pathogenesis of DED. We used standard mouse model of desiccating stress to induce DED. Then we assessed DED by Schirmer's test and clinical fluorescence scoring. Cell populations were quantified by myeloperoxidase assay, immunohistochemistry and flow cytometry. Lipid mediators were quantified by lipidomics and gene expression was quantified by qPCR. According to our investigation, female mice exhibited amplified dry eye severity compared to males. A population of resident PMN that is present in limbus, lacrimal gland and draining lymph nodes (DLNs) in both male and female mice prior to inducing DED, was marked decreased

after desiccating stress. This was coincident with the appearance of CD4⁺ T cells, especially in females. PMN depletion prior to inducing DED has reduced basal PMN by 66-84% in limbus, lacrimal gland and DLNs. The depletion resulted in a significant increase in CD4⁺ T cells after inducing DED. Lipoxin formation and pathway expression demonstrating activity of the circuit was significantly impaired in PMN depleted mice. These results provide first evidence that corneas, lacrimal glands and DLNs generate immune-regulatory LXA₄ depending on resident-regulatory PMN. Depletion of PMN results in impaired LXA₄ formation and amplified T cell response. These findings identify a novel regulatory role for PMN and LXA₄ in controlling lymphocyte activation in the pathogenesis of DED and ocular health of females.

IRB Status: Approved

Disclosures:

YUAN GAO, MD, PHD: No financial relationships to disclose

O132

HLA-A*0206 WITH TLR3 POLYMORPHISM EXERTS MORE THAN ADDITIVE EFFECTS IN COLD MEDICINE-RELATED STEVENS-JOHNSON SYNDROME WITH SEVERE OCULAR COMPLICATIONS

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Background: Stevens-Johnson syndrome (SJS) is an acute inflammatory vesiculobullous reaction of the skin and mucosa, often including the ocular surface, and toxic epidermal necrolysis (TEN) occurs with its progression. We previously reported that HLA-A*0206 with Toll-like receptor 3 (TLR3) Single-nucleotide polymorphisms (SNPs) exerts more than additive effects in SJS/TENS with severe ocular complications. In the present study we focused on only cold medicine related SJS/TEN and examined the multiplicative interaction between HLA-A*0206 and TLR3 SNP (rs.3775296), which show strongly more than additive effects in SJS/TENS with severe ocular complications.

Materials and methods: We analyzed the genotypes for HLA-A and TLR3 SNP (rs.3775296) in 88 Japanese cold medicine related SJS/TEN patients with severe ocular complications, which include 38 acetaminophen related SJS/TEN and 206 healthy volunteers to examine the interaction between the two loci.

Results: We found that for cold medicine related SJS/TEN, HLA-A*0206 exhibited a high ratio of carrier frequency: $p=4.0 \times 10^{-12}$, OR=6.7), and there was a strong association with TLR3 rs. 3775296 SNP (TLR3 rs3775296 TT vs TG+GG: $p=3.3 \times 10^{-4}$, OR=4.7), and that for acetaminophen related SJS/TEN, HLA-A*0206 also exhibited a high ratio of carrier frequency: $p=8.7 \times 10^{-11}$, OR=6.2), and there was a strong association with TLR3 rs. 3775296 SNP (TLR3 rs3775296 TT vs TG+GG: $p=8.5 \times 10^{-5}$, OR=6.6). The results of interaction analysis showed

that the pair, HLA-A*0206 and TLR3 SNP rs3775296T/T exerted more than additive effects ($p=1.4 \times 10^{-5}$, OR=49.4) for cold medicine related SJS/TEN, and that it also exerted more than additive effects ($p=5.1 \times 10^{-4}$, OR=53.9) for acetaminophen-related SJS/TEN.

Conclusion: The pair HLA-A*0206 and TLR3 SNP rs3775296T/T might be a marker which could predict the onset of SJS/TEN with severe ocular complication after taking cold medicine.

IRB Status: Approved

Disclosures:

MAYUMI UETA, MD, PHD: No financial relationships to disclose

O133

MOLECULAR MECHANISMS OF IMMUNE PRIVILEGE OF THE CORNEAL ALLOGRAFT

MACHIKO SHIMMURA-TOMITA

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The eye is the immune privileged organ, and corneal transplantation is the most successful organ transplantation. The molecular mechanisms of immune privilege have been learned from ocular inflammation animal models, especially corneal transplantation. At present, three major mechanisms prevail regarding the molecular mechanisms of immune privilege in the corneal transplantaion: there are 1) anatomical, cellular, and molecular barriers in the eye; 2) eye-derived immunological tolerance, the so-called anterior chamber-associated immune deviation (ACAID); and 3) immune suppressive intraocular microenvironment.

In this session, the functions of several molecules on local immune regulation within the cornea are reviewed. One of the topics in our recent study is T cell immunoglobulin and mucin domain (Tim)-3 and galectin (Gal)-9-mediated local immune regulation of corneal allograft. Gal-9 is constitutively expressed on the corneal epithelium, endothelium and iris-ciliary body in normal mouse eyes and the eyes bearing the surviving allografts, and that Tim-3-expressing cells were present in the allografts from the early period after grafting. Allograft survival in the recipients treated with anti-Tim-3 monoclonal antibodies (mAb) or anti-Gal-9 mAb are significantly shorter than that in the control recipients. In vitro, destruction of corneal endothelial cells by allo-reactive CD4⁺ T cells was enhanced when the cornea was pretreated with anti-Gal-9 mAb. The pathway of Tim-3/Gal-9 does not affect ACAID. Thus, Gal-9 expressed in the cornea plays a local immunosuppressive role in the corneal allografts, by protecting corneal endothelial cells from destruction by allo-reactive CD4⁺ T cells.

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Disclosures:

MACHIKO SHIMMURA-TOMITA, MD: No financial relationships to disclose

O134

SOX9 AND PPAR GAMMA AS POTENTIAL REGULATORS OF CORNEAL EPITHELIAL DIFFERENTIATION

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Transcription factors play important roles in cellular differentiation programs and have been used to guide stem cells towards specific lineages. We examined the potential involvement of SOX and PPAR transcription factors in limbal epithelial stem cell maintenance and corneal epithelial differentiation.

RNA from human basal limbal and corneal epithelial cells was obtained by combining laser capture microdissection (LCM) and mRNA-amplification. Quantitative real-time PCR (qPCR) was used to perform a complete screen of SOX and PPAR transcription factor expression in these specimens (n=5) and in cultured limbal epithelial cells at various stages of differentiation. Immunofluorescence was used to localize SOX8, -9, -10 and PPAR γ on cryosections of corneolimbal tissue both under resting and wound healing conditions. Changes in mRNA expression of potential target genes after transcription factor activation/overexpression and after siRNA-mediated knock-down were analyzed by real time PCR.

Genes of the SoxE group, particularly SOX9, and PPAR γ were consistently detected at higher levels in limbal epithelial cells compared to corneal epithelial cells obtained by LCM. Immunofluorescence showed preferential localization of SoxE factors to nuclei of limbal basal and suprabasal cells, whereas PPAR γ displayed a predominantly cytoplasmic expression pattern in limbal basal cell clusters. SOX9 and PPAR γ proteins co-localized with putative markers of limbal progenitors. Activation of wound healing in organ cultured corneas led to increased staining for SOX9 and PPAR γ across limbal and central corneal epithelial cells. In vitro, expression of both SOX9 and PPAR γ increased under differentiation-inducing conditions. Following siRNA-mediated downregulation of SOX9 and PPAR γ mRNA, cultured limbal epithelial cells downregulated expression of Keratin 15 and upregulated expression of Keratin 3. Conversely, activation of the PPAR γ pathway by glitazones induced upregulation of Keratin 15 and Keratin 12, whereas expression of Keratin 3 was downregulated.

IRB Status: None

Disclosures:

FRIEDRICH KRUSE, MD: No financial relationships to disclose

O135

LRIG1 CONTROLS CORNEAL MAINTENANCE THROUGH THE STAT3-DEPENDENT INFLAMMATORY PATHWAY

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DOSHISHA UNIVERSITY

Corneal integrity and transparency are indispensable for good vision. Homeostasis of the cornea is entirely dependent upon corneal stem cells. Epithelial damage triggers complex wound-healing processes that work to restore tissue homeostasis. The findings of this present study show that Lrig1 was highly expressed in the holoclone-type corneal epithelial stem cell population, and that Lrig1 was sporadically expressed in the basal cells of ocular-surface epithelium to regulate corneal epithelial cell fate during wound repair. Loss of *Lrig1* resulted in impaired recruitment of stem cells post wounding, and in the cell-fate switch from transparent epithelium to keratinized skin-like epidermis, leading to corneal blindness. Lrig1 controlled corneal cell fate during repair by negatively regulating the Stat3-dependent inflammatory pathway, and blocking Stat3 rescued the pathological phenotypes observed in *Lrig1* KO corneas. Additionally, expression of constitutively active Stat3 in the corneal epithelium of transgenic mice resulted in the abnormal features with corneal plaques and neovascularization that closely resemble those of *Lrig1* KO mice. Bone marrow chimera experiments indicated that Lrig1 may also coordinate the function of bone-marrow-derived inflammatory cells. Here, we demonstrate that Lrig1 orchestrates corneal-tissue transparency and cell fate during repair, and shed new light on the novel function of Lrig1 as a key regulator of tissue homeostasis.

IRB Status: Approved

Disclosures:

TAKAHIRO NAKAMURA, MD, PHD: No financial relationships to disclose

O136

INHIBITION OF TGF β SIGNAL TRANSDUCTION FACILITATES LIMBAL EXPLANT OUTGROWTH CULTURE IN DEFINED, XENO-FREE MEDIUM

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ICAHN SCHOOL OF MEDICINE AT MOUNT SINAI, OPHTHALMOLOGY

Expanded population of limbal epithelial precursor cells is needed to treat limbal stem cell deficiency (LSCD). Optimal expansion is achieved by explant culture or under the support of 3T3 mice fibroblasts in high calcium or under low calcium (non physiological condition) with bovine pituitary extract. In all these cases the medium requires animal fluid complementation. The purpose of this study was to assess whether the inability to expand the cells in serum-free medium is due to proliferative arrest and/or acceleration of differentiation caused by endogenously-generated TGF β . Rabbit limbal segments were set on explant culture. Culture was performed in, a) SHEM (DMEM/F12 complemented with, growth and nutrient factors and 5 % FBS); b) DMEM/F12 plus 16 % FBS (D/F16); and c) FBS-free SHEM (SFSHEM, defined xeno-free medium). The effect of the TGF β R-SMAD2/3 inhibitor SB431542 on early and late explant outgrowth rate, and on cell size, ABCG2-dependent JC-1 exclusion (IOVS, 52:4330) and colony formation indexes (CFI) of the outgrowth cell population were studied. SB431542 had minimal stimulatory (0-20%) effect on growth rates in SHEM but doubled the slower rate observed in D/F16

to equalize the SHEMA rate. In SHEMA an initial outgrowth became unproductive within the first 4-6 days due to continuous cell shedding at the edge of the outgrowth. SB431542 reversed this process and allowed continuous large expansion of the outgrowth, albeit at a slower rate than in SHEMA, in particular at the start of the explant culture. The inhibitor reduced average cell size and increased CFE in the three media and increased the average mitochondrial membrane potential. We conclude that TGF β is a major impediment in limbal epithelial cell culture and that inhibition of the canonical TGF β R pathway allows generation of high density limbal epithelial cultures under full xeno-defined media conditions.

IRB Status: None

Disclosures:

J. MARIO WOLOSIN, PHD: No financial relationships to disclose

O137

SEEING AROUND THE CORNER AT FUTURE STRATEGIES IN CORNEAL REGENERATION

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Corneal blindness affects 12 million people with 1.2 million new cases each year, and has profound personal and social costs for patients and their families. Corneal blindness is avoidable, and due to associated high financial cost, it has become an important public health concern.

Corneal regeneration using therapies derived from limbal stem cells or replacement through transplantation of donor corneas, are the most common modes of overcoming corneal blindness. Unfortunately, current strategies are greatly restricted in terms of limited clinical success, global availability, and cost. More cost and clinically effective alternatives are therefore urgently needed.

Mesenchymal stem cells (MSC) are the subject of intensive research for regenerative medicine purposes, as they possess a potent functional-repertoire. Whilst they can be differentiated into a steadily growing number of cell types, including those found in the cornea, their regenerative abilities extend beyond differentiation. They secrete a profile of potent trophic factors that promotes wound healing and tissue regeneration whilst suppressing localised immune responses. MSC encourage graft acceptance and reduce occurrences of GVHD/rejection, without long-term incorporation into the host tissue.

MSC have been found to reside in the corneal stroma. Although their role in normal corneal wound healing has yet to be fully elucidated, there is increasing evidence to suggest a potent allogeneic therapeutic potential for generic corneal regeneration.

This presentation will focus on challenges of corneal regenerative strategies, and the limitation of current solutions. New and emerging innovative treatment strategies will then be discussed, focusing on the developments in stem cell engineering at University of Nottingham.

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IRB Status: International

Disclosures:

ANDREW HOPKINSON, PHD: Equity Owner relationship with NuVision

O138

ALTERATION IN LACRIMAL GLAND CELLULAR TURNOVER AND PROGENITOR CELL POPULATION IN A MOUSE MODEL OF DRY EYE

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SCHEPENS EYE RESEARCH INSTITUTE, MASSACHUSETTS EYE AND EAR¹; BOSTON UNIVERSITY SCHOOL OF MEDICINE, OPHTHALMOLOGY²

We determined if a change in lacrimal gland (LG) cell turnover and progenitor cell population is associated with the development of dry eye in the thrombospondin (TSP)-1^{-/-} mouse model of dry eye. All experiments were performed on female wild type (WT) and TSP-1^{-/-} mouse LG at 12 weeks of age. H&E stained sections were evaluated by NIH Image J to determine cell size. Quantitative RT-PCR was used to determine the expression of IFN- γ , IL-1 β , IL-17, IL-6, and TNF α in LG homogenate. Cell proliferation was determined using BrdU injections and analysis by microscopy. Apoptosis was determined by flow cytometry using Annexin-V and propidium iodide. Amount of progenitor cell markers was determined by Western blotting and immunofluorescence microscopy. Lacrimal gland cell size and acinar area were smaller in TSP-1^{-/-} compared to WT mouse LGs. No cytokine expression was detected in either TSP-1^{-/-} or WT LGs. Cell proliferation was decreased and early apoptosis increased in TSP-1^{-/-} compared to WT LG. The transport protein and stem cell marker ABCG2 expressed in multiple types was increased in TSP-1^{-/-} compared to WT LGs. ABCG2 was extensively expressed in acinar cell basolateral membranes. ABCG2 does not appear to be a useful stem/progenitor cell marker in the LG. Neuronal stem cell markers Musashi1 and Sox2 were decreased in TSP-1^{-/-} compared to WT mice. The neural stem cell marker nestin and PAX6, CHX10, and an epithelial stem cell marker deltaNp63 were unchanged. All progenitor cell markers were expressed in the myoepithelial cells of LGs of TSP-1^{-/-} and WT mice. We conclude that in TSP-1^{-/-} mice, LG cellular deterioration and loss are initiated prior to inflammation and are accompanied with a change in stem cell population. The changes in LG homeostasis and inadequacy of specific progenitor cell population may contribute to dry eye in TSP-1^{-/-} mice.

IRB Status: None

Disclosures:

DARLENE DARTT, PHD: No financial relationships to disclose

CORNEAL STEM CELL-DERIVED GOBLET CELLS AND WNT SIGNALING PATHWAY

AHDEAH PAJOOHESH-GANJI, Sonali Pal-Ghosh, Gauri Tadvalkar, Mary Ann Stepp

THE GEORGE WASHINGTON UNIVERSITY

The pathology that appears on the mouse cornea after 2 mm circular corneal debridement wound involves the formation of keratin-8+ compound niches similar to those seen in corneal stem cell deficiency. These compound niches consist of cells in 3 different states: 1) mature cells identified by Muc5ac, 2) proliferating cells identified by Ki67, and 3) label-retaining cells identified by BrdU and β 4 integrin. They give rise to goblet cell clusters on the corneal surface only after large wounds (2 mm and more in diameter), which involve more cell proliferation, and not after small wounds (1.5 mm and less). We hypothesize that proliferation impacts the differentiation of corneal goblet cells. Epithelial differentiation is characterized by changes in expression of keratins and activation of the Wnt signaling pathway. To better understand the events that lead to the presence of corneal goblet cells, we assessed the expression of various keratins and Wnt-signaling factors within the corneal epithelium of Balb/C mice using QPCR, confocal microscopy, and immunoblots as a function of time after wounding. QPCR analyses show upregulation of K12 and downregulation of K14 genes at 1 and 2 days after wounding. The mRNA expression was similar to control at 4 and 6 weeks after wounding. Furthermore, GSK3 α , GSK3 β , and cyclin D are downregulated at the same time points. Immunoblotting showed a decrease in β -catenin 2d after wounding. Taken together our results indicate that Wnt signaling pathway is downregulated after 2mm wounding, which may be the reason for the presence of the goblet cell clusters on the mouse ocular surface. Therefore, activating Wnt signaling pathway early after wounding may prevent the formation of goblet cell clusters and corneal opacity.

IRB Status: None

Disclosures:

AHDEAH PAJOOHESH-GANJI, PHD: No financial relationships to disclose

INSULIN-LIKE GROWTH FACTOR-1 SUPPORTS DIFFERENTIATION AND TRANSDIFFERENTIATION OF STEM CELLS INTO CORNEAL-LIKE EPITHELIAL CELLS

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A proper function of the cornea is ensured by a population of limbal stem cells (LSC). We found that insulin-like growth factor-1 (Igf-1) plays an important role in differentiation of LSC into corneal epithelial cells. Igf-1, which is highly expressed in the cornea after the corneal injury, migrates to the limbus

where it binds to its receptor and triggers the differentiation process of LSC. If the damage involves limbal region, the only effective treatment of LSC deficiency is transplantation of LSC. However, LSC therapy has limitations in a low number of available LSC. Therefore, other sources of autologous stem cells are needed. Mesenchymal stem cells (MSC) with their immunomodulatory effects and differentiation potential can be used for targeted differentiation into corneal epithelial-like cells.

We established a mouse model of corneal damage and the central cornea was excised at various time intervals. As we observed, the expression of genes expressed in the cornea, such as keratin 12 (K12), keratocan (Kera) and lumican (Lum) was highly decreased after corneal damage. Therefore we prepared the predifferentiated corneal-like cells which can be used as a source of cells suitable for transplantation on the damaged corneal surface. Murine MSC were cultured with the extract prepared from the excised corneas in the presence or absence of the recombinant Igf-1. Analysis by qPCR showed that after 10 days of MSC differentiation the expression of corneal specific markers (K12, Kera, Lum) was significantly increased. When Igf-1 was used with the extract, the expression of these genes was further increased. Microscopic analysis of predifferentiated MSC was also performed by fluorescence staining for K12 protein.

The results suggest that Igf-1 is not only responsible for the differentiation of LSC into corneal epithelial cells, but also supports targeted differentiation of MSC into corneal epithelial-like cells.

IRB Status: None

Disclosures:

PETER TROSAN, MSC: No financial relationships to disclose

THE LANTS, A NEW FAMILY OF EXTRACELLULAR REGULATORS OF LIMBAL EPITHELIAL BEHAVIOUR

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UNIVERSITY OF LIVERPOOL, EYE AND VISION SCIENCE

Recently, we described the identification of a new family of extracellular matrix proteins, the LaNts, which share similarities with laminins and netrins. Using novel mouse monoclonal antibodies against one family member, LaNta31, we demonstrate that this protein is expressed in human limbal and corneal epithelium with the distribution pattern changing from basement membrane associated in the limbal region to intracellular in the corneal epithelium. In limbal-derived keratinocytes in culture, LaNta31 displays a punctate distribution beneath cells, is enriched at cell peripheries, particularly beneath actin based projections. Through a combination of knockdown and exogenous addition studies we have analyzed the functional roles of LaNta31. Keratinocytes displaying knockdown in LaNta31 attach less rapidly and less robustly compared to scrambled siRNA controls. Additionally, knockdown keratinocytes display defective motility with reduced migration velocities in low-density migration assays and slower scratch wound closure rates. Consistent with these data, the expression of LaNta31 at both the protein and mRNA level is increased following introduction of a scratch wound. Exogenous addition of purified LaNta31 enhances the migration

rates of keratinocytes at low-density and promotes the early stages of scratch wound closure. Based on its domain architecture and localization, we hypothesize that LaNta31 may interact with laminins and influence their function. Consistent with this, in vitro laminin polymerisation assays demonstrate that addition of LaNt leads to increased polymer formation. Functionally, addition of purified LaNt reduced the rate of attachment of corneal epithelial cells to laminin-111 but had no effect on attachment to collagens I, IV or fibronectin. Together these data indicate important functional roles for a new family of extracellular matrix proteins, establish that cellular responses to structural components of the extracellular matrix can be modulated by the action of small linker proteins and specifically implicates the LaNts as being important in regulating wound healing.

IRB Status: Approved

Disclosures:

KEVIN J. HAMILL, PHD: No financial relationships to disclose

CO04 – Corneal Stromal and Endothelial Biology

O142

THE BIOLOGICAL CHARACTERISTICS OF HUMAN CORNEAL ENDOTHELIAL CELLS

SHIGERU KINOSHITA

KYOTO PREFECTURAL UNIVERSITY OF MEDICINE

Understanding the biological characteristics of human corneal endothelial cells (HCECs) is essential for corneal endothelial disease-associated translational research. To date, our efforts have focused on attempted to develop new therapeutic modalities for the treatment of corneal endothelial diseases such as Fuchs' endothelial corneal dystrophy. In our initial attempt, we used cultivated CECs for the treatment of advanced corneal endothelial dysfunction. We are currently investigating the clinical application of a novel 'cell-injection therapy' using cultured HCECs combined with the application of Rho-associated protein kinase (ROCK)-inhibitor Y-27632 into the anterior chamber. We discovered that the use of ROCK-inhibitor Y-27632 and the inhibitor of the transforming growth factor-beta signaling pathway successfully maintains a normal phenotype of serially cultured HCECs without epithelial mesenchymal transition. The other attempt of our research is focused on developing a novel medical treatment for the early phase of corneal endothelial disease. To that end, our findings show that the use of ROCK-inhibitor eye drops is effective for treating partial endothelial dysfunction, as CEC migration and proliferation is accelerated and thus results in the production of a newly formed CEC layer. We hope that our present and future findings will help promote the development of novel CEC-based therapies for the treatment of corneal endothelial dysfunction.

IRB Status: Approved

Disclosures:

SHIGERU KINOSHITA, professor: No financial relationships to disclose

O143

OXIDATIVE DNA DAMAGE IN FUCHS ENDOTHELIAL CORNEAL DYSTROPHY

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MASSACHUSETTS EYE AND EAR INFIRMARY / HARVARD MEDICAL SCHOOL

The corneal endothelium is a highly metabolically active tissue and is known for its high abundance of mitochondria. Fuchs endothelial corneal dystrophy (FECD) is a progressive disease in which there is a loss of corneal endothelial cells leading to corneal edema and eventually corneal blindness. The goal of our study is to examine the effect of oxidative stress on DNA damage in FECD. We detected at baseline a 8.5-fold and 10.4-fold lower mitochondrial DNA (mtDNA) copy number in FECD cell line (FECDi) as compared normal CE cell lines (HCEEnC and HCECi), indicating mitochondrial destruction in FECD. At baseline, FECDi exhibited a 77% ($p=0.009$) and 89% ($p=0.0001$) decrease in mtDNA and nDNA relative amplification, respectively, compared to normal cell lines. Moreover, FECDi displayed a significantly higher mtDNA (1.5 lesions/10kb, $p=0.001$) and nDNA (1.9 lesions/10kb, $p=0.005$) lesion frequency (or DNA damage) compared to normal cell lines (zero-class lesion frequency). Treatment with Menadione (MN), a pro-oxidant, decreased mtDNA and nDNA amplification by 77% ($p=0.02$) and 78% ($p=0.02$), respectively in FECDi compared to normal cell lines. In addition, mitochondrial membrane potential (MMP) was on average 2.8-fold lower ($p=0.05$) in FECDi compared to normal cell lines at baseline. Exposure to MN further decreased MMP in FECDi as compared to normal cells suggesting presence of mitochondrial dysfunction. Treatment with MitoQ, a mitochondria-targeted antioxidant, protected FECDi cell line from damage caused by MN exposure. Loss of MMP in FECDi correlated with the decline in cellular ATP levels, increase in reactive oxygen species (ROS) production, and apoptosis, indicating oxidative stress-induced decline in mitochondrial function in the diseased cells. This data indicate that chronic accumulation of oxidative DNA damage leads to mitochondrial dysfunction and apoptosis, and contributes to the endothelial cell degeneration seen in FECD.

IRB Status:

Disclosures:

ADNA HALILOVIC, PHD: No financial relationships to disclose

O144

NOVEL STRATEGIES FOR ENGINEERING HUMAN CORNEAL ENDOTHELIUM BY HC-HA/PTX3 REGENERATIVE MATRIX FROM AMNIOTIC MEMBRANE AND P120 CATENIN-KAISO SIGNALING

SCHEFFER TSENG, Ying-Tieng Zhu

TISSUETECH, INC.

Currently there is no medical treatment for corneal blindness caused by dysfunctional human corneal endothelial cells

(HCECs), a clinical problem inflicting estimated 39 million people in 2012 by the World Health Organization. Because the only treatment resorts to transplantation of healthy donor HCECs, tissue engineering is one option to solve global shortage of donor corneas. Nonetheless, the conventional engineering of HCECs based on single cells derived from EDTA-trypsin treatment that disrupts cell-cell junctions runs the risk of irreversible endothelial mesenchymal transition by activating canonical Wnt and TGF- β signaling. Herein, we successfully engineer one HCEC monolayer of 11.0 ± 0.6 mm in diameter with a normal phenotype using the Descemet's membrane stripped from 1/8 corneal rim normally discarded after routine corneal transplantation. This method does not disrupt cell-cell junctions but activates RhoA-ROCK-canonical BMP signaling to reprogram adult HCECs to neural crest-like progenitors via activation of the nuclear miR 302b-Oct4-Sox2-nanog complex. Using HC-HA/PTX3 purified from amniotic membrane to suppress TGF- β signaling and promote BMP signaling, we are able to reprogram human corneal fibroblasts to neural crest progenitors that can then be induced to HCECs. Collectively, these new strategies disclose a new possibility of treating degenerative diseases by unlocking the hidden proliferative potential through reprogramming not all the way to iPSCs.

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IRB Status: Verified

Disclosures:

SCHEFFER TSENG, MD, PHD: Employee relationship with Tissue-Tech

O145

ROLES OF TRP CHANNELS IN SMAD SIGNAL AND MYOFIBROBLAST FORMATION IN STROMAL REPAIR

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TRP family members are nonselective ion channels that are activated by specific ligands or temperatures. TRPV1 or TRPA1 is one of the members and is activated by capsaicin and heat over 42°C, or by a variety of chemical and physical stimuli including pungent agents, temperature, pain, lipid, pH and shear stress, respectively. In cornea TRPV1 or TRPA1 is expressed in epithelial cells and sensory nerve ends and also upregulated in corneal fibroblasts upon injury. Loss of TRPV1 or TRPA1 inhibited activation of TGF β -dependent signal transduction, i.e., Smad, JNK, and p38 and also counteracted myofibroblast formation and fibrogenic growth factor upregulation in vitro. In alkali burn model in cornea lacking either TRPV1 or TRPA1 suppressed inflammatory and fibrogenic responses, i.e., macrophage invasion and myofibroblast generation, during wound healing, and subsequently provided a healing with more transparent stroma, as compared with wild-type mice. Gene expression analysis showed loss of each receptor reduced inflammatory/fibrogenic growth factor expression. Systemic administration of chemical inhibitors for these receptors

reproduced the favorable anti-inflammatory/fibrogenic effects of knockout phenotypes. TRPV1 or TRPA1 is a potential therapeutic target for the purpose of suppression of inflammatory/fibrogenic events in cornea.

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SHIZUYA SAIKA, MD, PHD: No financial relationships to disclose

O146

INTERRUPTION OF WNT/ β -CATENIN SIGNALING AXIS IN KERATOCYTES CAUSES PRECOCIOUS CORNEAL EPITHELIUM STRATIFICATION VIA BMP4

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UNIVERSITY OF CINCINNATI, OPHTHALMOLOGY¹; CHUNG-GUNG MEMORIAL HOSPITAL, OPHTHALMOLOGY²

Mesenchyme-epithelial interaction plays pivotal roles on corneal morphogenesis during development. Conditional ablation of β -catenin (*Ctnnb1*) in corneal stromal keratocytes (*Ctnnb1^{cko}*) triggers precocious epithelium stratification in mice. In this study, attempts were made to explore the mechanisms controlling this aberrant developmental event. To investigate the role of Wnt/ β -catenin signaling axis on corneal morphogenesis, *Ctnnb1* and *Lrp5/6* genes were ablated in keratocytes of KR/TC/*Ctnnb1^{fl}* and *KR/TC/Lrp5^{fl}/Lrp6^{fl}* compound mice by Doxycycline induction from embryonic day 12.5 (E12.5) through P0 (day of birth) or P0 through P10. Histochemical examination indicated that *Lrp5cKo/Lrp6cKo* double knock-out mice manifested precocious corneal epithelium stratification similar to the phenotype observed in *Ctnnb1^{cko}* mice, while single *Lrp5^{cko}* or *Lrp6^{cko}* mice were normal. Primary cultured corneal fibroblasts of *Ctnnb1^{fl}* were infected with *Ad-Cre* or *Ad-EGFP* virus. Analysis of cDNA micro-array, RT-PCR and western blotting showed that BMP4 was up-regulated in cultured corneal fibroblasts from *Ctnnb1cKo* mice. The effect of BMP4 on precocious corneal epithelium stratification was verified by BMP4-treated neonates that displayed phenotype resembling *Lrp5^{cko}/Lrp6^{cko}* and *Ctnnb1^{cko}* mice. Furthermore, Immunofluorescence staining revealed that MAPK pathway was activated via activation ERK1/2 by phosphorylation in HTCE cells treated with BMP4. Thus, the precocious corneal epithelium stratification observed in *Ctnnb1* and *Lrp5/6* mutants may result from the up-regulated expression of BMP4 in developing corneal stroma via the activation MAPK, i.e., phosphorylation of ERK1/2. Our data supports the notion that cross-talk between Wnt/ β -catenin/BMP4 axis (in stroma) and BMP4/ERK1/2 signaling (in epithelium) play critical roles in corneal morphogenesis during development.

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IRB Status: None

Disclosures:

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INVOLVEMENT OF TRANSFORMING GROWTH FACTOR BETA IN EXTRACELLULAR MATRIX DEPOSITION AND ENDOPLASMIC RETICULUM STRESS IN FUCHS' CORNEAL DYSTROPHY

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The pathogenesis of Fuchs' endothelial corneal dystrophy (FECD) has yet to be elucidated. We recently reported that epithelial-to-mesenchymal transition (EMT)-related genes are involved in FECD (Koizumi N, ARVO 2014). The purpose of this study was to evaluate the involvement of transforming growth factor beta (TGF- β) signaling in the production of extracellular matrix (ECM) and endoplasmic reticulum (ER) stress in FECD corneal endothelial cells (CECs). FECD-patient human CECs (HCECs) and normal donor-cornea HCECs were cultured and immortalized to produce iFECD and iHCEC cellular models. To determine ECM protein synthesis and production, expression of collagen type I, fibronectin, and protein disulfide isomerase (ER-marker) were analyzed by immunostaining. To assess morphological changes of cell organelles, iFECD and iHCEC were investigated by transmission electron microscopy (TEM). To evaluate ER stress, ER stress-sensor (IRE1, PERK, and ATF6) expression was analyzed by Western blotting of iFECD and iHCEC with or without TGF- β stimulation. To elucidate involvement of the TGF- β signaling pathway in apoptosis, Annexin V-positive apoptotic cells were evaluated by flow cytometry following TGF- β treatment. Collagen type I and fibronectin, which could be immunolocalized to the ER, were expressed at higher levels in iFECD than in iHCEC. TEM demonstrated that ER and mitochondria were dilated in iFECD, but appeared normal in iHCEC. TGF- β significantly increased the production of ECM associated with the expression of IRE1, PERK, and ATF6 in iFECD in comparison to iHCEC. In addition, TGF- β significantly increased Annexin V-positive cells in iFECD compared to untreated control ($29.9 \pm 1.5\%$ and $19.4 \pm 1.4\%$, respectively), while the percentage of Annexin V-positive cells was not increased by TGF- β stimulation in iHCEC ($12.3 \pm 0.5\%$ and $11.1 \pm 0.6\%$, respectively). Our findings suggest that TGF- β induces excessive production of ECM associated with ER stress in CECs in FECD. The TGF- β signaling pathway may therefore provide a potent therapeutic target for treating FECD.

IRB Status: Approved

Disclosures:

NAOKI OKUMURA, MD, PHD: Patents/Royalties relationship with Doshisha university

CORNEAL STROMAL SCARRING: TARGETING THE DEUBIQUITINASE USP10 PREVENTS INTEGRIN-MEDIATED FIBROSIS

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ICAHN SCHOOL OF MEDICINE, MOUNT SINAI, OPHTHALMOLOGY

Fibrotic disease results from the persistence of myofibroblasts, cells that excessively contract tissue and secrete disorganized extracellular matrix. Stromal myofibroblasts are characterized by excess cell surface expression of integrin $\alpha 5 \beta 1$ that participates in generating fibrosis through increased cell adhesion and by direct activation of the fibrotic growth factor, TGF β . Genetic screening of myofibroblasts by RNAseq revealed increased gene expression of a subset of deubiquitinating enzymes (DUBs). DUBs remove ubiquitin from specific substrate proteins, reducing their degradation, resulting in a net increase in protein levels. Recent work reveals that inhibiting DUBs reduces aberrant disease-causing protein accumulation offering a novel potential therapeutic intervention. Over-expression of the DUB USP10 induced a significant increase in total and cell surface integrin $\alpha 5 \beta 1$ protein, 2.0 and 3.3-fold, respectively. The increase resulted from decreased degradation of internalized integrin $\alpha 5 \beta 1$ and the recycling of non-degraded integrin to the cell-surface. Cell-surface accumulation of integrin $\alpha 5 \beta 1$ led to a 2-fold increase in the activation of endogenous TGF β , and the induction of downstream fibrotic markers: α -SMA organized into stress fibers and secretion and extracellular assembly of cellular fibronectin (FN-EDA). In corneal ex-vivo organ culture after wounding, stromal myofibroblasts (α -SMA) appeared concomitantly with a significant increase (2-12 fold) in USP10, integrin $\alpha 5 \beta 1$, and FN-EDA protein expression. Genetic silencing of USP10 after wounding prevented the induction of these fibrotic markers. This work demonstrates for the first time a connection between DUBs and myofibroblasts and suggests that USP10 may be a novel anti-fibrotic target.

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IRB Status: Verified

Disclosures:

AUDREY BERNSTEIN, PHD: No financial relationships to disclose

CO05 – Corneal Development, Differentiation, and Genetics

INTERACTION BETWEEN HEDGEHOG SIGNALING, RETINOIC ACID AND PAX6 DURING CORNEAL REGENERATION

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UNIVERSITY OF ABERDEEN¹; UNIVERSITY OF EDINBURGH²

Corneal wound healing is regulated by multiple partially redundant signaling systems, including those mediated by Hedgehog-ligand signaling. In this study, it was found that corneal epithelial wound healing rates increased in response to applica-

tion of Sonic hedgehog ligand (Shh), but only in mice with wild-type Pax6 dosage. Mice heterozygous for Pax6, which show an ocular surface degeneration phenotype, increased corneal epithelial proliferation in response to exogenous Shh but did not show an accelerated wound healing response. Downregulation of Hedgehog signaling was found to completely inhibit corneal epithelial cell proliferation. Desert hedgehog (Dhh) was shown to be the major endogenous ligand, with Shh detectable only at very low levels, and only after epithelial wounding. The activity of phosphatidylinositol-3-OH kinase- γ (PI3K γ) was not required for the increased migration response in response to Shh. Pax6 $^{+/-}$ Gli3 $^{+/-}$ double heterozygotes showed highly disrupted patterns of clonal arrangement of cells in the corneal epithelium. The mechanism by which increased hedgehog signaling accelerates wound healing and proliferation in corneal epithelia was investigated. It was found that application of topical Shh polypeptide to corneal epithelia activated the retinoic acid response pathway. The data show key roles for endogenous Dhh signaling in maintenance and regeneration of the corneal epithelium, demonstrate an interaction between Pax6 and Hh signaling, and show that failure of Hh signaling pathways is a feature of Pax6 $^{+/-}$ corneal disease that cannot be remedied pharmacologically by addition of the ligands.

IRB Status: International

Disclosures:

J MARTIN COLLINSON, MA, PHD: No financial relationships to disclose

O150

FGF AND WNT SIGNALING AND THE HOMEBOX TRANSCRIPTION FACTOR, OTX1, PATTERN THE OCULAR SURFACE EPITHELIA AND REGULATE THE EXPRESSION OF SIX2 AND SIX3

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The sine oculis-related homeobox transcription factors, Six2 and Six3 are well known key regulators of kidney development, lens induction, retinal specification and brain development. We found that Six2 and Six3 are expressed in complementary regions of the ocular surface epithelium at early stages of mouse eye development (E13.5). Six3 transcripts were detected in the prospective central corneal epithelium and Six2 was selectively expressed in the prospective conjunctival epithelium. Fgf10 signaling through Fgfr2 is required for lacrimal gland formation (Makarenkova et al. 2000. Development). Expression of Six2 was lost when Fgfr2 was deleted from the ocular surface using the Le-Cre transgene, suggesting that conjunctival differentiation, not just lacrimal gland formation, is dependent on FGF signaling. Otx1 is required for the formation of the ciliary epithelium and for normal brain development and is expressed in the ocular surface epithelial cells under the control of Pax6. In embryos in which Otx1 was deleted or in which canonical Wnt signaling was prevented by conditional deletion of the Wnt co-receptors, Lrp5 and 6, Six3 expression extended from the corneal into the conjunctival epithelium. Ectopic expression of Six3

in the conjunctival epithelium correlated with the expression of Keratin-12 (K12) in the prospective conjunctiva, suggesting that Otx1, Fgf and Wnt signaling prevent the conjunctiva from becoming cornea by suppressing the expression of Six3 and K12. We obtained Six2 knockout mice and will use them to define its role in the formation of the conjunctival epithelium. We also have Six3 floxed mice and are testing the role of Six3 in the specification of the corneal epithelium. These studies provide new information about the signaling pathways and transcription factors responsible for the regional specification of the ocular surface epithelia.

IRB Status: None

Disclosures:

JIE HUANG, PHD: No financial relationships to disclose

O151

FUCHS ENDOTHELIAL CORNEAL DYSTROPHY: GENETICS, PATHOGENESIS, AND POTENTIAL NON-SURGICAL TREATMENT

ALBERT JUN

WILMER EYE INSTITUTE, JOHNS HOPKINS MEDICAL INSTITUTIONS

Fuchs endothelial corneal dystrophy (FECD) is a common cause of corneal vision loss. The definitive treatment is keratoplasty, and FECD is a leading indication for this surgery. Mutations in genes including SLC4A11, TCF8, CLU, TGFBI, LOXHD1, and AGBL1 have been associated with FECD. The majority of cases are associated with a trinucleotide repeat expansion in the TCF4 gene. Mutations in the COL8A2 gene cause an early onset form of FECD. Using a variety of biophysical and molecular approaches in addition to studies of cell and animal models and human tissue, we have identified processes associated with FECD pathology. Analysis of two different COL8A2 mutations show altered collagen triple helical thermal stability and intracellular accumulation. Human tissues and Col8a2 mutant mouse models of FECD show endoplasmic reticulum (ER) stress and unfolded protein response. Tissue microarray analysis of FECD tissues indicates upregulation of cyclin dependent kinase inhibitors p16 and p21 supporting a role for senescence. MicroRNA (miRNA) profiling of human tissues showed global downregulation. In particular, reduced expression of three members of the miRNA-29 family correlated with increased levels of extracellular matrix components collagen-I and -IV and laminin which are characteristic features of FECD pathology. Based on its potential anti-oxidant and anti-ER stress effects, oral lithium was assessed for potential to modify the FECD phenotype in Col8a2 mutant mice. Lithium treated FECD mice showed a 25% ($p < 0.01$) increase in endothelial cell density compared to untreated mice. Lithium treatment showed significant increases ($p < 0.05$) in endothelial expression of autophagy markers P62, Tmem74, Tm9sf1, and Tmem166. Thus, a wide range of pathologic processes affect the endothelium in FECD, and further study is needed to elucidate their relationship to each other and to primary events leading to cell death. Results from lithium treated FECD mice validate the potential to modify the disease phenotype using medical approaches.

IRB Status: Approved

Disclosures:

ALBERT JUN, MD, PhD: Patents/Royalties relationship with Johns Hopkins University

O152**GENETICS OF CORNEAL THICKNESS****XIAOYI GAO**

UNIVERSITY OF ILLINOIS

Central corneal thickness (CCT) is associated with vision disorders including keratoconus and glaucoma. Multiple genome-wide association studies (GWAS) including meta-analyses, most of which were conducted using subjects of European and Asian ancestry, have reported many genetic loci associated with CCT. However, it is a significant challenge to prioritize single nucleotide polymorphisms (SNPs) and refine the associated GWAS signals since most of the hits are intronic or intergenic. In recent years, several advances in genetics have improved the process. For example, GWAS using Latinos have enabled researchers to interrogate the genetic loci under a different linkage disequilibrium structure from Europeans and Asians. Genotype imputation based on the 1000 Genomes Project (1KGP) reference panels has allowed researchers to probe far denser genomic coverage than the GWAS originally genotyped. Information from the ENCODE (Encyclopedia of DNA Elements) project has demonstrated that a majority of GWAS signals are concentrated in regulatory regions. In this talk, I will show that by combining information from GWAS of Latino subjects, 1KGP genotype imputation, ENCODE evidence, and gene expression using human ocular tissues, the annotation of SNPs that regulate CCT can be greatly improved.

IRB Status: Approved

Disclosures:

XIAOYI GAO, PHD: No financial relationships to disclose

O153**NOVEL MEMBRANE-TETHERED MUCINS AT THE OCULAR SURFACE AND IN THE AQUEOUS OUTFLOW PATHWAY**

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The glycocalyx at the ocular surface provides for hydration and lubrication, and prevents pathogen access to the underlying tissue. We showed that multipurpose contact lens solutions increase risk for corneal infection by destroying the protective membrane-tethered mucin layer (Gordon et al., 2011, IOVS 52:9188). Ocular surface epithelia are known to

express three membrane-tethered MUC-type mucins: MUC1, -4, and -16. A novel mucin gene cluster was recently identified within the major histocompatibility (MHC) locus on human chromosome 6 (Hijikata et al., 2011, Hu Genet 129:117), which includes the genes encoding two new MUC family members: MUC21 and MUC22. We show by PCR that corneal epithelial cells express both MUC21 and MUC22. Immunostaining demonstrates that MUC22 is expressed in the upper layers of the epithelium at the human ocular surface. The protein encoded by MUC21 has been previously characterized (Itoh et al., 2008, Glycobiology 18:74), but MUC22 is novel. Conceptual translation indicates that MUC22 encodes a large membrane-tethered protein of 174 kDa. Transient expression of a cloned variant of MUC22 cDNA with a truncated mucin repeat region (57 kDa) tagged with green fluorescent protein (27 kDa) enriches in the plasma membrane, migrating at ~200 kDa on westerns. The pro-inflammatory cytokine IL-1 stimulates expression of MUC22, while TGF-beta or glucocorticoids inhibit basal and IL-1-stimulated expression. Interestingly, MUC22 is also expressed in trabecular meshwork cells of the eye's aqueous outflow pathways. We will discuss possible roles for the novel mucins at the ocular surface and in the aqueous outflow pathways.

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Disclosures:

M. ELIZABETH FINI, PHD: No financial relationships to disclose

O154**MOUSE GENOMIC LOCI MODULATING CENTRAL CORNEAL THICKNESS**

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Central corneal thickness (CCT) is a highly heritable ocular trait that impacts several human disorders including primary open angle glaucoma (POAG). We have used a mouse genetic reference panel (the BXD RI strain set) to define mammalian genomic loci modulating CCT with a total of 339 mice from 63 BXD RI strains (between 60-100 days of age). The mice were deeply anesthetized and the eyes were positioned in front of the Mouse Bore of a BiopTigen ocular contrast tomography (OCT) system. CCT data for each strain was averaged and used to identify quantitative trait loci (QTLs) modulating this phenotype using the bioinformatics tools on GeneNetwork (www.genenetwork.org). This analysis reveals one significant QTL on Chr 13 and several suggestive QTLs on Chr 6, Chr 16 and Chr 19. The significant locus on Chr 13 was examined further to define potential candidate genes modulating this eye phenotype. The 9 mb region under the Chr 13 peak in the mouse

distributes over 4 different chromosomes in the human: Chr 1, Chr 6, Chr 7 and Chr 10. Using the eye and retina microarray datasets (HEIMED and HEI Retina) on GeneNetwork, we identified 16 genes with cis-QTLs that could be modulating CCT in the BXD RI strain set. These 16 genes are on Chr 13 between 9 and 22 mb and include *C7orf10*, *Mplkip*, *Cdc2l5*, *Stard3nl*, *Elmo1*, *Sfrp4*, *Zscan12*, *Zkscan3*, *Zfp187*, *H4k*, *H2b*, *H4fe*, *Nkapl*, *Trm27*, *Nfrf*, *Stard3* and *Sfrp4*. These candidate genes were examined to determine if they are potential risk factors for human glaucoma using meta-data from the NEIGHBOR-GLAUGEN GWAS. Several SNPs located in an intragenic region near *ELMO1* demonstrated nominal significance ($p = 0.001$ for lead SNP rs9986865). This approach can identify candidate gene modulating CCT in the mouse and a potential risk factor for primary open angle glaucoma.

IRB Status: None

Disclosures:

ELDON GEISERT, PHD: No financial relationships to disclose

O155

USING MOUSE GENETICS TO IDENTIFY QTL ASSOCIATED WITH CENTRAL CORNEAL THICKNESS

DEMELZA KOEHN, Michael Anderson

UNIVERSITY OF IOWA

Purpose: Central corneal thickness (CCT) is a continuously distributed, quantitative trait in humans and mice. CCT is relatively stable within individuals over time, but varies widely between different individuals and ethnicities, and varies widely among inbred mouse strains. CCT is also one of the most highly heritable human traits, with heritability estimates reported up to 0.95. Despite the fact that CCT is easy to clinically record, important to human disease, and highly heritable, relatively few genetic variants influencing it have thus far been identified. To complement efforts to identify important CCT-loci in humans, we are using mouse genetics to map quantitative trait loci (QTL) that influence CCT in mice.

Methods: Different strains of inbred mice were intercrossed and backcrossed to produce a population of F2 and N2 progeny, respectively. Mice from both crosses were analyzed for the CCT phenotype using optical coherence tomography (OCT) and genotyped using a genome-wide polymorphic SNP panel. Genotype-phenotype associations were made using R/qtl. A set of diversity outbred mice (DO/J) were analyzed for the CCT phenotype using OCT.

Results: F2 progeny from the intercross mice identified two significant QTL that regulate CCT on chromosome 7. N2 progeny from the backcross mice identified a significant QTL on chromosome 11 and a suggestive QTL on chromosome 19. The set of DO/J mice displayed a continuous distribution of CCT, ranging from 80.5 μm to 120 μm .

Conclusions: Using crosses of mice from different inbred lines, we have uncovered several QTL responsible for controlling the magnitude of CCT and are currently mapping the genes underlying these QTL. We have also shown that the recently

developed diversity outbred mice will be a useful resource for identifying CCT-regulating QTL.

IRB Status: None

Disclosures:

DEMELZA KOEHN, PHD: No financial relationships to disclose

CO06 – Corneal Innervation: Lesson from the Basic Science

O156

IN VIVO VISUALISATION OF MURINE CORNEAL NERVE FIBRE REGENERATION IN RESPONSE TO CILIARY NEUROTROPHIC FACTOR

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UNIVERSITY OF ROSTOCK, DEPARTMENT OF OPHTHALMOLOGY

Investigations in the mouse model addressed the regeneration capabilities of the subbasal nerve fibre plexus (SNP), and the influence of local ciliary neurotrophic factor (CNTF) application on the regeneration process. In preliminary experiments, the healthy mouse cornea was monitored using *in vivo* confocal laser-scanning microscopy (CLSM), to reveal and rule out the age-dependent changes in SNP. No changes in nerve fibre density NFD were detected in untreated animals over time; mean NFD in mice aged 8 weeks ($28.30 \pm 9.12 \text{ mm/mm}^2$), 16 weeks ($29.23 \pm 7.28 \text{ mm/mm}^2$), 30 weeks ($26.31 \pm 8.58 \text{ mm/mm}^2$) and 52 weeks ($26.34 \pm 6.04 \text{ mm/mm}^2$). For regeneration studies a circular incision through corneal epithelium and anterior stroma of minimum 60 μm depth was generated to cut the subbasal corneal nerves in adult mice. The corneal nerve pattern was monitored and NFD was measured before and up to 8 weeks after surgery. The CNTF group received eye drops containing CNTF (25ng/ml) 3 times daily for 3 weeks, whereas the control group received no further medication. In the sham group the same treatment schedule was applied as in CNTF group, using vehicle. All three groups revealed a marked NFD reduction starting at one week after incision, followed by continuous recovery. After 8 weeks the NFD reached $23.5 \pm 2.4 \text{ mm/mm}^2$ (78% of baseline), $21.9 \pm 1.6 \text{ mm/mm}^2$ (73% of baseline) and $29.2 \pm 3.4 \text{ mm/mm}^2$ (93% of baseline) in the control, sham and CNTF group, respectively. By comparison with control and sham group, the CNTF group demonstrated significantly higher NFD at every observation time point. The mouse cornea provides a practicable animal model for *in vivo* CLSM monitoring of corneal nerve behaviour over time. Non-penetrating trephination generated a severe reduction in the NFD of the SNP, but murine corneas recovered to pre-injury NFD levels within 8 weeks. Local application of CNTF served merely to temporarily accelerate the recovery of NFD.

IRB Status: International

Disclosures:

OLIVER STACHS, PHD: No financial relationships to disclose

THE REGENERATIVE AND INFLAMMATORY BIOACTIVITY OF PIGMENT EPITHELIAL DERIVED FACTOR (PEDF) AND DOCOSAHEXAENOIC ACID (DHA) AFTER CORNEAL NERVE DAMAGE

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DEPARTMENT OF OPHTHALMOLOGY AND NEUROSCIENCE CENTER

Loss of corneal innervation induces decrease in corneal sensitivity and persistent inflammation, and it can lead to corneal ulceration. Herpes simplex virus (HSV-1) is a clinically common infection with consequences of inflammation and scarring of the cornea and severe nerve damage. Our laboratory have shown that PEDF plus DHA treatment increase nerve regeneration after experimental lamellar keratectomy. The aims of the present study were: a) To evaluate the action of PEDF plus DHA treatment in a rabbit model infected with HSV-1, and b) To investigate the action of two bioactive smaller PEDF derivatives in association with DHA in corneal nerve regeneration after experimental surgery.

In a), rabbits were infected with 17Syn+ HSV-1 strain and treated with PEDF plus DHA. After 1 and 2 weeks of HSV-1 post-infection, vehicle-treated corneas showed a high number of inflammatory cells. Treatment with PEDF plus DHA significantly decreased inflammation and the severity of corneal lesions. Sensitivity was recovered up to 9 weeks and a significant increase in nerve regeneration was observed in corneas treated with PEDF plus DHA. In b), an 8mm corneal stroma dissection was performed in the left eye of rabbits and treatment groups received PEDF, 34-mer PEDF or 44-mer PEDF, all plus DHA, for 6 weeks. Eight weeks after refractive surgery, there was a significant increase in corneal sensitivity and nerve density in the 44-mer PEDF plus DHA- treated rabbits compared to the 34-mer PEDF plus DHA and vehicle.

Our results show that PEDF plus DHA treatment has anti-inflammatory actions and stimulates regeneration of nerves seriously damaged by HSV-1 infection. The 44-mer PEDF could be a novel therapeutic agent with greater bioavailability and more easily synthesized than the complete PEDF molecule for treating neurotrophic keratitis that develops as a result of corneal nerve damage.

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IRB Status: Approved

Disclosures:

HAYDEE BAZAN, PHD: No financial relationships to disclose

MYELOID CELL-MEDIATED NERVE REGENERATION – LESSONS LEARNED AND TRANSLATIONAL ASPECTS

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The mechanisms responsible for corneal nerve regeneration are not fully determined, but they constitute a high priority in the field of ophthalmology. We investigated the hypothesis that myeloid cells that traffic in the cornea have neuroregenerative actions. The presence of fluorescent myeloid cells in the thy1-YFP transgenic mouse provides a powerful tool for in situ visualization of interactions between the nervous and inflammatory (myeloid) systems. Therefore, in this study, we characterized fluorescent bone marrow cells (BMCs) of the thy1-YFP mouse and determined their action on trigeminal ganglion (TG) cell neurite growth. Excimer laser annular keratectomy was performed and corneas were imaged. BMCs were harvested from femur and tibia, and the expression of surface markers on YFP+ BMCs was analyzed by flow cytometry. The immunosuppressive action of BMCs (YFP+ and YFP-) was evaluated in an allogenic mixed lymphocyte reaction (MLR). Neurotrophic action of BMCs (YFP+ and YFP-) was determined in compartmental and transwell cultures of dissociated TG cells. Following annular keratectomy, YFP+ BMCs infiltrated the cornea. YFP+ BMCs shared surface markers (CD11b+Gr1+Ly6C+Ly6G-F4/80low) with monocytic myeloid-derived suppressor cells (MDSCs), had similar morphology, and suppressed T-cell proliferation in allogenic MLR in a dose-dependent manner. YFP+ BMCs, but not YFP- BMCs, significantly increased growth of TG neurites in vitro. When cultured in a transwell with TG neurites, YFP+ BMCs expressed neurotrophins and secreted nerve growth factor (NGF) in conditioned medium. YFP+ BMCs that infiltrated the cornea maintained their phenotype and actions (neuronal and immune). These data show that YFP+ BMCs in thy1-YFP mice have immunophenotypic features of MDSCs. They secrete NGF and promote neuroregeneration. These findings increase our understanding of the beneficial roles played by leukocyte trafficking in the cornea and may lead to therapeutic strategies that use NGF-secreting myeloid cells to repair diseased or injured neurons.

IRB Status: None

Disclosures:

SANDEEP JAIN, MD: No financial relationships to disclose

FROM THE CORNEA TO THE BRAIN AND BACK: A NOVEL ROLE FOR CORNEAL NERVES

GIULIO FERRARI

SAN RAFFAELE SCIENTIFIC INSTITUTE

The cornea receives the densest innervation of the whole body. Corneal sensory nerves originate in the trigeminal ganglion (TG), and reach the cornea through the first (ophthalmic) branch of the trigeminal nerve. Corneal nerve terminals exert a number of functions beyond mere sensory perception; these include support for epithelial cell proliferation and/or migration and possibly immune regulation. Effects of trigeminal nerve dysfunction/ablation on the cornea have been described and are clinically known as neurotrophic keratopathy. However, the effect of corneal nerve damage and/or ablation on TG has not been clarified yet.

In order to test whether a corneal injury may induce inflammation in the trigeminal ganglion, we used the mouse corneal alkali burn model. Following alkali burn of the cornea, a significant CD45+ leukocyte infiltration in the right TG at 4 and 8 days was observed. In vivo MRI with a macrophage tracking contrast agent showed increased uptake in the right TG, which peaked at day 8. Macrophages were observed histologically in the right TG and exhibited a M2-phenotype (CD45+ F4/80+ CD206+). Additionally, pro-inflammatory cytokines, including Substance P and NK-1R were significantly increased in the TG after alkali burn. Finally, the expression of IL-1 and VEGF-A was significantly reduced in the right TG after topical treatment with dexamethasone.

We suggest, for the first time, inflammatory involvement of brain structures following ocular surface damage. Our findings support the hypothesis that the neuropeptide Substance P may be involved in the propagation of inflammation from the cornea to the TG through corneal nerves.

IRB Status: Approved

Disclosures:

GIULIO FERRARI, MD: No financial relationships to disclose

O160

FAILED REINNERVATION AT SITES OF CORNEAL EROSIONS

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THE GEORGE WASHINGTON UNIVERSITY¹; THE GEORGE WASHINGTON MEDICAL SCHOOL, ANATOMY AND REGENERATIVE BIOLOGY²

Axons from the trigeminal ganglion innervate the cornea entering at the limbal region. As they track towards the center of the cornea forming a spiral pattern, numerous branches of the subbasal nerves innervate the apical surface of the cornea. We hypothesize that the tonic release of vesicles by the nerves may play trophic roles and provide growth factors to the corneal epithelial cells. When the cornea is injured, the fine subbasal nerves are either crushed or severed and must be regenerated from the stumps at the wound edge. The density of axons and temporal aspects of reinnervation have not been elucidated. Using BALB/c mice and whole mount confocal imaging, we follow the reinnervation of the subbasal nerves at various time points (5h, 18h, 24h, 5d, and 4w) in two animal models: 1) debridement wounding using the dulled blade where the basement membrane is left behind and 2) debridement wounding using rotating burr where the basement membrane is removed. Data indicate that while a small fraction of the subbasal nerves move along under the leading edge during reepithelialization, most reinnervation takes place after 24h and the subbasal nerve density remains decreased 4w after injury compared to unwounded controls. Erosions begin to form between 1 and 2 weeks in many of the dulled blade wounded corneas. The majority of corneas show a circular area with fewer or no subbasal nerves remaining on the cornea at 5d and 4w. Sites of poor reinnervation persist in corneas without erosions. These data suggest that reinnervation

defects and erosion formation are correlative. However, further studies are required to discern whether reinnervation defects arise before or after or erosion formation.

IRB Status: None

Disclosures:

MARY ANN STEPP, PHD: No financial relationships to disclose

O161

CORNEAL NERVE ACTIVITY AND OCULAR SENSATIONS UNDER PATHOLOGICAL CONDITIONS

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The ocular surface is richly innervated by the peripheral nerve fibers of trigeminal ganglion neurons. Although nerve fibers reach the cornea, conjunctiva and eyelids as thin myelinated or unmyelinated nerve fibers without morphological terminal specialization, they are functionally heterogeneous based upon their response to specific stimuli. Mechanonociceptor fibers (~20% of the total) respond only to mechanical forces; polymodal nociceptor fibers (~65-70%) respond to mechanical forces and also to heat, exogenous chemical irritants and endogenous inflammatory mediators; cold thermoreceptor fibers (~10-15%) increase markedly their firing frequency in response to moderate cooling. These differences in transduction capacity among ocular sensory fibers are attributable to the variable expression of different types of transduction channels.

Selective activation of the different types of sensory nerve fibers using gas esthesiometry evokes specific qualities of conscious sensations referred to the eye that include always a variable component of unpleasantness. In addition, sensory fibers play a role in the trophic maintenance of the ocular surface and the modulation of basal and reflex tearing.

Under pathological conditions (inflammation, chronic dryness of the ocular surface, surgical injury) activity of ocular sensory nerve fibers changes markedly due to short-term changes in ion channel activity and/or expression induced by local inflammatory agents and growth factors, and long-lasting modifications in gene expression. These changes lead to the development of spontaneous activity and of abnormal responsiveness to natural stimuli of ocular nerve fibers, leading to changes in the quality of the experienced sensation in humans, and also to disturbances in tearing, blinking and ocular surface trophism.

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IRB Status: Approved

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CORNEAL SENSITIVITY LOSS IN PTERYGIUM PATIENTS: A COMPARATIVE ANALYSIS BETWEEN AFFECTED AND UNAFFECTED ZONE

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Pterygium is a common ocular surface lesion originates from altered limbal stem cells, which proliferate into the cornea, accompanied by degradation of Bowman's layer and elastosis. Corneo-conjunctival changes could trigger tear film alterations that, in some conditions, reduce corneal sensitivity. The purpose of this study was to compare corneal sensitivity on affected and unaffected zone, thus assessing a relative loss of sensitivity.

Thirty-nine eyes of 39 patients with primary nasal pterygium were included in this study. Cochet-Bonnet aesthesiometer were used to evaluate corneal sensitivity of 3 points on pterygium lesion (delimiting a triangle) and 4 points on unaffected cornea (central, temporal, upper and lower). All the measurements were made for the same practitioner and in similar conditions of humidity and temperature. Results on one zone were added to calculate the sensitivity of this zone.

Abnormal sensitivity in the three point of the lesion was found in 35 eyes (89.74%) being the mean sensitive value of the affected zone 13.8 ± 2.8 cm (normal mean value of 18 cm, since the zone include 3 points and normal result is 6 cm in each point). Mean sensitivity in unaffected cornea was 23.5 ± 1.0 cm (normal mean values of 24 cm). Four eyes showed normal mean values in the two zones. A significant sensitivity reduction in the affected zone was found when results of the two zones were compared ($p < 0.001$; Wilcoxon test) (data were normalized to avoid influence of the different number of points analyzed in each zone). Pterygium changes seem to induce corneal sensitivity loss, regardless changes in tear film.

IRB Status: Approved

Disclosures:

PAMELA CAMPOS FIGUEROA, OPHTHALMOLOGY RESIDENT PHYSICIAN: No financial relationships to disclose

CO07 – Corneal Angiogenesis from Translational Aspects

MMPS AND THEIR INHIBITION IN CORNEAL NEOVASCULARIZATION

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UNIVERSITY OF ILLINOIS AT CHICAGO

Corneal extracellular matrix (ECM) remodeling by MMPs has been implicated in corneal angiogenesis and in the maintenance of corneal avascularity. MMPs are a group of

zinc-binding proteolytic enzymes that participate in ECM remodeling, NV, and lymphangiogenesis. The upregulation of MMPs has been demonstrated to occur during corneal angiogenesis. However, their definitive roles in the regulation of angiogenesis are ambiguous because the same molecule can simultaneously act as a pro-angiogenic and an anti-angiogenic factor. The dual function of MMPs during angiogenesis may be explained by their ability to degrade the ECM, allowing tissue invasion by MMP-bearing endothelial cells, and to generate or release anti-angiogenic fragments from their precursors. Understanding MMP functions is necessary to devise translational strategies for ocular therapies.

IRB Status: None

Disclosures:

DIMITRI AZAR, MD, MBA: Board of Directors relationship with Novartis

ANTILYMPHANGIOGENESIS IN CORNEAL TRANSPLANTATION AND DRY EYE DISEASE

CLAUS CURSIEFEN

DEPARTMENT OF OPHTHALMOLOGY, UNIVERSITY OF COLOGNE

The normal cornea is devoid of both blood and lymphatic vessels. Nonetheless under several pathological conditions ingrowths of blood and lymphatic vessels can occur into the cornea. Pathological lymphangiogenesis has been implicated in mediating corneal graft rejection as well as ocular surface inflammation such as in dry eye disease. Current evidence supporting this concept and novel therapeutic options by modulating immune responses by targeting lymphangiogenesis are discussed. Current clinical trials on antilymphangiogenic therapy are evaluated.

IRB Status: Accepted

Disclosures:

CLAUS CURSIEFEN, MD: No financial relationships to disclose

MECHANISMS OF BLOOD VESSEL REGRESSION

PAT D'AMORE

SCHEPENS EYE RESEARCH INSTITUTE, MASSACHUSETTS EYE AND EAR, HARVARD MEDICAL SCHOOL

The development, stabilization and regression of the microvasculature have been the subject of extensive investigation. Paracrine (soluble factors), juxtacrine (cell-associated factors) and cell-cell interactions have been identified as mechanisms regulating these processes. The actions of VEGF, PDGF B, TGF β and the angiopoietins are orchestrated for the formation of a stable, mature microvessel. Nascent vessels that do not undergo remodeling to recruit pericytes and produce a basement

membrane are more prone to regression. This feature is, in fact, central to the concept of anti-angiogenic therapeutics, which takes advantage of the fact that newly formed, immature vessels are particularly dependent on VEGF. However, mature vessels are not "terminally differentiated", that is, they appear to require trophic factors for their continued maintenance and can be induced to further proliferate. The nature and degree of the requirement for trophins appears to differ among vessel types, with fenestrated vessels particularly dependent on the continued presence of VEGF. Although molecules that mediate lymphangiogenesis have been identified, less is known about the mechanisms that mediate the stabilization of small lymphatic vessels. In contrast to capillaries, small lymphatics lack pericyte investment and have an incomplete basement membrane so it would appear unlikely that the specific mechanisms that mediate capillary stabilization apply to lymphatic stabilization/regression. Interestingly, there is evidence for a macrophage-dependent formation of transient lymphatics during inflammation.

IRB Status: Not provided

Disclosures:

PAT D'AMORE: No financial relationships to disclose

O166

TUMORS OF THE OCULAR SURFACE AND ANGIOGENESIS

BERTIL DAMATO

U.C. SAN FRANCISCO OPHTHALMOLOGY AND RADIATION ONCOLOGY

Angiogenesis is one of the hallmarks of cancer. The aims of this presentation are to review angiogenesis in the three most common ocular surface malignancies: conjunctival melanoma, conjunctival squamous cell carcinoma, and extraocular spread of uveal melanoma. Conjunctival melanomas and squamous cell carcinomas usually originate in the epithelium before becoming invasive. Interestingly, intra-tumoral lymphatics are already present in tumors confined to the epithelium, such vessels becoming more abundant as invasion occurs. The degree of lymphangiogenesis is greater when these tumors show other risk factors for local recurrence and metastasis and lymphatic density is itself an independent risk factor for these outcomes. For example, conjunctival melanomas are more likely to metastasize if located medially and it is known that lymphatic density is greater in medial conjunctiva. In contrast, uveal melanomas show no lymphatics unless extraocular spread has occurred. It has been suggested that lymphatic density may be a useful prognostic indicator in these tumors and further studies are indicated. There may be scope for including this feature in the scoring system for conjunctival melanocytic intra-epithelial neoplasia. It has been suggested that anti-angiogenic therapy may be useful in treating conjunctival malignancies as well as preventing local recurrence and even metastatic disease. The surest ways of achieving these objectives include tumor excision, topical chemotherapy and adjunctive radiotherapy; however, further studies would be required before anti-angiogenic agents to determine clinical efficacy in patients not responding to conventional forms of treatment.

IRB Status: None

Disclosures:

BERTIL DAMATO, FRCO, MD, PHD: No financial relationships to disclose

O167

EFFECTS OF VEGF DEPLETION ON CORNEAL NERVES

MARK ROSENBLATT, Victor Guaiquil, Zan Pan, Natalia Karagianni, Shima Fukuoka, Gemstonn Alegre

WEILL CORNELL MEDICAL COLLEGE, NEW YORK

In addition to its potent effects as a pro-angiogenic molecule, vascular endothelial growth factor (VEGF) has been shown to be a potent stimulator of nerve survival and growth. Anti-angiogenic therapy using anti-VEGF antibodies is a burgeoning treatment modality for the treatment of corneal neovascularization, but could conceivably cause diminished corneal innervation.

We have examined the role of VEGF inhibition in regulating corneal innervation in vitro and as well as in intact and injured cornea. Our studies demonstrate VEGF isoforms play a role in mediating corneal nerve repair, but do not alter baseline innervation. Thus, anti-VEGF therapy may decrease re-innervation, but has little effect on baseline innervation. The use of anti-VEGF therapy may require close monitoring in the setting of neuropathic corneas.

IRB Status: Not provided

Disclosures:

MARK ROSENBLATT: No financial relationships to disclose

O168

INTEGRIN ALPHA 9 BLOCKADE INHIBITS LYMPHATIC VALVE FORMATION AFTER CORNEAL TRANSPLANTATION

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UNIVERSITY OF CALIFORNIA AT BERKELEY

Lymphatic dysfunction is associated with many pathologic conditions from transplant rejection to cancer metastasis. To date, there is still a lack of effective treatment for lymphatic diseases. The cornea provides an ideal tissue for lymphatic research due to its unique features. We have recently provided evidence showing that luminal lymphatic valves are formed during corneal inflammatory lymphangiogenesis (LG) via the up-regulation of integrin alpha 9 (Truong et al. PLOS ONE, 2011), and these structures play a critical role in directing lymphatic drainage inside the vessels (Truong et al. IOVS, in press). The purpose of this study is to further determine whether integrin alpha 9 blockade would interfere with the process of lymphatic valve formation induced by corneal transplantation. Orthotopic corneal transplantation was performed between fully mismatched C57BL/6 (donor) and BALB/c (recipient) mice. Recipients were randomized to receive subconjunctival injections of either integrin alpha 9 or isotype control antibody twice a week for 8 weeks before

whole-mount corneas were harvested for immunofluorescent microscopic analysis. Digital images were analyzed by NIH Image J software to quantify lymphatic valve and vessel distribution ($p < 0.05$ was considered significant). Our results showed that the number of lymphatic valves was significantly reduced in the integrin alpha 9 treatment group. Moreover, the ratio of valves to lymphatic invasion area was also significantly lower in this treatment group. Additionally, we found that corneal lymphatic vessels induced by transplantation were predominantly distributed in the nasal than the temporal quadrant. In conclusion, this study shows that it is possible to suppress lymphatic valve formation via integrin alpha 9 interference. Further investigation on the integrin alpha 9 pathway may provide novel strategies to treat ocular as well as systemic lymphatic diseases, such as inflammation and transplant rejection.

IRB Status: None

Disclosures:

LU CHEN, MD, PHD: No financial relationships to disclose

CO08 – Novel Corneal Imaging Technologies from Basic Science to Clinical Application

O169

MODALITIES OF MULTIPHOTON IMAGING IN VIVO

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LEIBNIZ UNIVERSITAET HANNOVER¹; FRIEDRICH-SCHILLER-UNIVERSITAET JENA, INSTITUTE OF APPLIED OPTICS²; UNIVERSITY OF ROSTOCK, DEPARTMENT OF MEDICAL BIOCHEMISTRY AND MOLECULAR BIOLOGY³

Laser scanning microscopy and two-photon microscopy are useful tools for in vivo observation of the structures within the anterior chamber of the eye, especially the cornea. With the possibility of label-free characterization of tissues by second-harmonic or third-harmonic generation and the excitation of autofluorescence, cellular resolution within living specimens can be achieved. Further modalities, like polarization resolved excitation and detection allow further diagnosis of collagenous structure. In this area, we are using different laser polarizations, like radially or azimuthally polarized laser fields, to achieve analysis of the collagenous structure of the cornea. Using adaptive optics, consisting of a deformable mirror with 144 actuators and an open loop feedback algorithm, higher penetration depth and evaluation of the lens in the mouse model is enabled. Possible applications within the field of in vivo imaging, for example the characterization of nerve fiber damage in diabetic mice will be shown.

IRB Status: International

Disclosures:

ALEXANDER HEISTERKAMP, PHD: No financial relationships to disclose

O170

3-DIMENSIONAL ASSESSMENT OF THE CORNEAL RESPONSE TO INJURY AND DISEASE *IN VIVO* USING A MODIFIED HRT-RCM CONFOCAL MICROSCOPE

WALTER PETROLL, Danielle Robertson, H. Dwight Cavanagh

UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL CENTER, OPHTHALMOLOGY

We previously described hardware and software modifications for the Heidelberg Engineering Rostock Corneal Module (HRT-RCM) that allowed quantitative, full thickness 3-D scanning of the normal rabbit cornea *in vivo*. In this study we tested the capabilities of this system for assessing corneal injury and disease in both the rabbit and mouse. To automate the HRT-RCM focusing mechanism, a motor drive was attached to the microscope housing. Continuous scans were made from the endothelium to the epithelium while collecting images using HRT streaming software. Image sequences were decoded to allow calculation of their z-position using in-house software, and displayed interactively using our modified confocal microscopy through focusing (CMTF) program, which provides measurements of sub-layer thicknesses and estimates of stromal backscattering. For assessment of wound healing, rabbit and/or mouse corneas were scanned both before and after transcorneal freeze injury (FI) or mechanical debridement injury (MI). Following both FI and MI, inflammation and stromal edema was observed 1 day after injury. By 7 days, stromal cells repopulating the damaged tissue assumed an elongated and interconnected fibroblastic morphology, and a significant increase in cellular light scattering was measured. Stromal haze and edema gradually decreased as wound healing progressed. We also investigated the effects of hyperglycemia on corneal sub-layer thicknesses using a streptozotocin induced Type 1 diabetic mouse model. Interestingly, long-term hyperglycemia in the mouse was associated with both epithelial and stromal thinning. Epithelial thinning correlated with the loss of the sub basal nerve plexus measured *ex vivo*. Overall, the modified HRT-RCM allows high resolution 3-D image stacks to be obtained from both the rabbit and mouse cornea. These datasets can be used for interactive visualization of corneal cell layers, measurement of epithelial and stromal thickness, assessment of cell morphology and inflammation, and estimation of stromal backscatter (haze) in injury or diseased states.

IRB Status: None

Disclosures:

WALTER PETROLL, PHD: No financial relationships to disclose

O171

LARGE SCALE *IN VIVO* IMAGING OF THE CORNEAL SUB-BASAL NERVE PLEXUS

BERND KÖHLER¹, Stephan Allgeier¹, Susanne Maier¹, Klaus-Martin Reichert¹, Sabine Peschel², Rudolf Guthoff², Oliver Stachs², Georg Bretthauer¹

KARLSRUHE INSTITUTE OF TECHNOLOGY, IAI / AIA¹; UNIVERSITY OF ROSTOCK²

The densely innervated human cornea is the only superficial tissue of the human body in which nerve fibres are accessible in vivo by corneal confocal microscopy (CCM). Numerous studies were aimed at establishing morphometric features of the corneal sub-basal nerve plexus (SNP) as a sensitive marker for various ocular and systemic conditions and diseases. However, due to considerable variability in local nerve fibre density across the corneal area, evaluation of a single image with a typical field of view of 0.16 mm² is insufficient for reliable morphometric characterization. Mosaicking approaches have been proposed to examine the SNP on a larger scale. Here we present a technique that significantly facilitates the generation of mosaic images of the SNP by a high degree of automation. A computer-controlled moving fixation target on a display located in front of one eye guides the patient's gaze in a widening spiral pattern, while the other eye is being examined by CCM. Due to the synchronicity of human eye movements, the examined eye performs identical movement patterns below the microscope, which thus captures an image sequence of a continuously increasing area of the SNP. Specifically developed image processing software registers the sequence, removes motion artefacts, and fuses the acquired images by weighted averaging. The presented easy-to-use and fast technique for imaging extended areas of the SNP could pave the way for a more robust and reliable morphometric analysis of corneal nerve fibres with potential use in diabetic neuropathy diagnosis and provide new insight into the living human nervous system.

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IRB Status: International

Disclosures:

BERND KÖHLER, MD: No financial relationships to disclose

O172

IMMUNOFLUORESCENT TOMOGRAPHY OF OCULAR SURFACE ADULT STEM CELLS

GERAINT PARFITT, James Jester

UC IRVINE, GAVIN HERBERT EYE INSTITUTE

The precise location and number of adult stem cells in the different tissues that constitute the ocular surface is currently unknown. It is imperative to characterize adult stem cells in order to understand cell dynamics, turnover and age-related dysfunction of tissues. Immunofluorescent Tomography is a novel method that enables sequential immunostaining and the localisation and quantification of sub-populations of cells in a high-resolution 3-D volume. The purpose of this study was to use immunofluorescent tomography to reveal the position and quantity of label-retaining cells (LRCs) in the H2B-GFP transgenic mouse ocular surface.

Using the 'tet-off' system, GFP fluorescence in epithelial nuclei dilutes two-fold with every division after doxycycline chase. After long-term chase, GFP is retained in quiescent, putative epithelial stem cells only. Eyelids, corneas and lacrimal glands

were serially sectioned, imaged for GFP and sequentially immunolabeled for Ki67, Sox9, Blimp1 and keratin 5 and 6 to identify proliferating cells, putative stem cell markers and keratinised epithelia, respectively.

In quantitative 3-D reconstructions, keratin 6 immunostaining shows that LRCs are preferentially located at the terminal regions of ductal epithelium in the meibomian gland. Meibomian gland LRCs are Sox9⁺ and Blimp1⁻ unlike hair follicle LRCs which exhibit a varied expression of these putative stem cell markers. The lacrimal gland and limbal LRCs were both found to be Sox9⁺.

Because of improved axial resolution, immunofluorescent tomography offered unprecedented views into fluorescent gradients and revealed, for the first time, that a quiescent stem cell population may direct meibomian gland turnover. The presence of quiescent LRCs in the conjunctival fornix and lacrimal gland ductal epithelium suggests new sites of stem cell 'niches' that may dictate tissue turnover and potentially paves the way for novel regenerative therapies using these adult stem cells.

IRB Status: Verified

Disclosures:

GERAINT PARFITT, PHD: No financial relationships to disclose

O173

THREE-DIMENSIONAL MATRIX ULTRASTRUCTURE OF THE DEVELOPING CORNEA REVEALED BY SERIAL BLOCK FACE SCANNING ELECTRON MICROSCOPY

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CARDIFF UNIVERSITY, CARDIFF CENTRE FOR VISION SCIENCE¹; UNIVERSITY OF MANCHESTER, WELLCOME TRUST CENTRE FOR CELL AND MATRIX RESEARCH²

The cellular mechanisms by which the specialized matrix of the corneal stroma is formed in embryonic development are still not fully understood. Here, we report the use of serial block face scanning electron microscopy (SBF-SEM) to generate 3-D reconstructions of embryonic avian cornea to identify novel cell-matrix interactions which might be important for the formation of the ordered orthogonal matrix. Corneas were isolated from chick embryos at 10 and 18 days of incubation. Over this period, cells secrete orientated collagen fibril bundles which ultimately, from around day 12, condense into the organised lamellae of the mature stroma. SBF-SEM of stained and resin-embedded tissue revealed that keratocytes represented a proportion of the tissue which was greater than previously assumed, consistent at around 20% throughout. At day 12 these cells had complex morphology with extensive lamellipodia and filopodia-like protrusions, extending up to 100 microns from the cell surface. Interestingly, large numbers of aligned and uniformly spaced collagen fibrils were present in invaginations in the cell membrane, appearing as fibril bundles extending from the cell surface, and a distinct orthogonality in both cell and matrix organization, quantifiable by Fourier analysis, was evident. Three-di-

mensional reconstructions revealed that the actin-associated tubular cell protrusions, extended more than 30 μm into the extracellular space. Some appeared closely apposed to single collagen bundles, while others established contact with multiple bundles. The large cellular component of embryonic stroma was unappreciated previously because the extent of filopodia-like processes, we term keratopodia, was not fully evident in 2-D images. Keratopodia may represent cellular structures with unique dimensions and cornea specific-function, with a crucial role in orientation and organization of collagen into lamellae.

IRB Status: None

Disclosures:

ANDREW QUANTOCK, PHD: No financial relationships to disclose

O174

ADVANCED BRILLOUIN SPECTROSCOPIC TECHNIQUES

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The ability to determine biomechanical properties of the ocular tissue is a current challenge during the diagnosis and treatment of the anterior segment pathologies. The Brillouin-spectroscopy allows the spatial-resolved characterization of rheological and biophysical properties of transparent tissue. Our research focus is targeted on the transition from experimental approaches to clinical applications.

The main challenge of Brillouin-spectroscopy is to separate the inelastic Brillouin-signals from the elastic Rayleigh-signal. Therefore a new spectral filter was developed, which suppressed the intense elastic Rayleigh-signal completely and enables a spectral resolution of 2×10^{-5} nm (working wavelength $\lambda = 780.2456$ nm) by using a single VIPA set up. The developed filter technique in combination with a central-axial focusing enables an axial resolution of 10 nm. Using these configuration measurements of thin layers like the cornea is possible.

In conclusion, the Brillouin-spectroscopy is on the way to be a valid tool for evaluating the mechanical properties of the ocular anterior segment tissues like the cornea and eye lens.

IRB Status: International

Disclosures:

STEPHAN REISS, PHD: No financial relationships to disclose

CO09 – Corneal Tissue Engineering and Gene Therapy

O175

STRATEGIES OF HUMAN CORNEA TISSUE REGENERATION

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THE CATHOLIC UNIVERSITY OF KOREA

The corneal tissue is essential to maintain the transparency and vision, but several pathologies affecting these tissues lead to vision loss and eventual blindness.

Repair and reconstruction of the cornea has historically relied on corneal transplantation. But the donors' shortage, increased risk of immune rejection and the increased life expectancy contributed for the development of strategies to regenerate or repair ocular tissues. The cornea regeneration has been developed at an increasing pace and already provides benefits to regenerative medicine for cornea bio-replacement and stem cell-based therapies by reprogramming of its cells and tissue types with several approaches including the use of pharmacology, chemistry, cell biology, biomaterials, tissue engineering, gene therapy and stem cell transplantation.

IRB Status: None

Disclosures:

CHOUN-KI JOO, MD, PHD: No financial relationships to disclose

O176

BIOSYNTHETIC CORNEAS AS REGENERATION TEMPLATES: AN UPDATE

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There are 10 million corneal blind individuals worldwide, who are waiting for transplantation due to a shortage of donated corneas (Vision Share eyebank consortium estimates, 2010). There are also patients currently not transplanted because allografting is high risk and contraindicated. Our objective was to develop biosynthetic corneas that could in the future help address the shortage of donated corneas as well as the high risk transplantation. We demonstrated in a first-in-humans Phase I clinical study the regeneration of the human cornea (an organ that normally does not regenerate) using carbodiimide-crosslinked recombinant human collagen-based immune compatible implants. The regenerated neo-corneas have been stable over 4 years and have regenerated their different layers and nerves. Most importantly, our corneal implants have been stable over 4 years without immunosuppression and no rejection. Longitudinally-followed patients grafted with donor corneas showed one rejection

episode during the first year (11%; the average in Sweden is 15% within the first 2 years), despite use of immunosuppression during this time. We have now reinforced these implants by introducing a second polymer network, and have shown initial feasibility for transplantation in high risk cases where conventional allografting is contraindicated, e.g. in chemical burns to the cornea. In the future, biosynthetic corneas may be a viable supplement to the shortfall of donor corneas, and also a possible option for high risk grafts. However, clinical testing of the reinforced implants in a rigorous clinical trial will be needed to confirm this. We have, however, also shown feasibility of extension to more complex organ systems e.g. skin and cardiac repair, albeit in animal models.

IRB Status: International

Disclosures:

MAY GRIFFITH, PHD: No financial relationships to disclose

O177

BIO-PROSTHESIS: A BOTTOM-UP APPROACH TO CORNEAL TISSUE ENGINEERING

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UNIVERSITY OF READING, SCHOOL OF CHEMISTRY, FOOD AND PHARMACY

We developed a novel bioactive material able to support adhesion and proliferation of human corneal fibroblasts *in vitro* whilst emulating the cell's *in vivo* phenotype. Our biomaterial thus constitutes an easy-to-use, user-friendly surface template derived from functionalized peptide amphiphiles that instructs attached cells to maintain proper morphology while promoting the expression of specific components of the extracellular matrix, including collagen type-I. This allows cells isolated from human donors to survive for long periods in serum-free culture conditions, and consequently, to fabricate native-type tissues with highly-organized, 3D architectures. The biomaterial is also protease-sensitive, a property that allows it to be degraded by the end of the culture process and release the structurally-whole tissue with minimal manipulation. We envision that tissues fabricated *in vitro* using this technology will comprise a major source of live, modular, native-like tissue in the near future, with important applications in cell biology and tissue engineering modeling, as well as in regenerative medicine.

IRB Status: None

Disclosures:

CHE CONNON, PHD: No financial relationships to disclose

O178

SILK FOR CORNEAL TISSUE ENGINEERING

GILSON KHANG¹, Eun Young Kim¹, Nirmalya Tripathy¹, Jeong Eun Song¹, Choun-Ki Joo², Dongwon Lee²

CHONBUK NATIONAL UNIVERSITY¹; THE CATHOLIC UNIVERSITY OF KOREA²

There are lots of diseases related in corneal endothelium such as posterior polymorphous corneal dystrophy, congenital hereditary endothelial dystrophy, Fuchs endothelial corneal dystrophy and X-linked endothelial corneal dystrophy, etc. These results in loss vision through serial process according to losing corneal endothelium. Thus, they need corneal transplantation to restore corneal endothelium, finally. However, supplying donor cornea is limited, worldwide. So, studies about reconstructing tissue engineered substratum for corneal endothelium are focused on now. Natural based biomaterials are studying to delivery corneal endothelial cells and to use as a substratum. We focused on silk and fabricated silk fibroin film to fabricate film type substratum. Silk used as a medical suture has been applied for few centuries and is focused on various fields including corneal regeneration field. It has good biocompatibility and proper mechanical properties and can be prepared to transparent film. For these reasons, we choose silk fibroin as materials to prepare scaffold for corneal endothelium. Transparency, diverse mechanical properties, cultured morphology, initial density, proliferation assay, expression of specific marker and proteins were evaluated to confirm possibility as a carrier for corneal endothelium. Fabricated silk fibroin film is transparent and has proper mechanical properties to able to apply implant material. Silk fibroin film gives positive effects on attachment, spreading, and proliferation. Also, cultured corneal endothelial cells were maintaining specific morphology on silk fibroin film and functions, which is evidenced by initial density and expression of specific genes such as Na⁺/K⁺-ATPase, aquaporin-1, VDAC2 and 3, CLCN2, and NCB1 and proteins like ZO-1 and Na⁺/K⁺-ATPase. Thus, these results demonstrated that silk fibroin offers proper environment and can maintain to expression of their functions. That is, it can be a possible candidate for reconstructing corneal endothelium

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IRB Status:

Disclosures:

GILSON KHANG, MD, PHD: No financial relationships to disclose

O179

BIOMATERIALS FOR REPAIR AND REGENERATION OF EYE TISSUES

JENNIFER ELISSEFF, Qiongyu Guo, Shoumyo Majumdar, Jeremy Chae

JOHNS HOPKINS UNIVERSITY

Repair and reconstruction of the cornea has historically relied on synthetic materials or tissue transplantation. However, the future holds promise for treatments using smart biomaterials and stem cells that direct tissue repair and regeneration to ultimately create new ocular structures that are indistinguishable from the original native tissue. By understanding the physical structure of the tissue and the resulting impact of the structure on biological function, we can design novel materials for a number of ophthalmic clinical applications. We are pursuing synthetic, biopolymer, and tissue-based strategies employ-

ing materials science strategies to create biomimetic corneal implants. These materials include collagen membranes with aligned and organized fibers and vitrified tissues. Biocompatibility and cellular phenotype is characterized in the materials and physical properties defined to provide clarity and strength. In vivo testing is employed to evaluate regenerative properties. Finally, concepts of building an "eye-on-chip" will be presented.

IRB Status: Not provided

Disclosures:

JENNIFER ELISSEFF, PHD: No financial relationships to disclose

O180

OCULAR SURFACE RECONSTRUCTION WITH ORAL MUCOSAL EPITHELIAL CELL SHEETS

YOSHINOBU OIE, Kohji Nishida

OSAKA UNIVERSITY GRADUATE SCHOOL OF MEDICINE

Introduction: Oral mucosal epithelial cell sheets have been successfully used to reconstruct eyes with limbal stem cell deficiency (LSCD). This procedure can solve the problems of conventional limbal transplantation including long-term immunosuppression that involves high risks of serious eye and systemic complications, including infection, glaucoma, and liver dysfunction. However, cell sheets must be fabricated in a Cell Processing Center (CPC) under Good Manufacturing Practice conditions for clinical use, and the expenses of maintaining a CPC are too high for all hospitals to cover. Therefore, several hospitals should share one CPC to standardize and spread the application of regenerative therapy using oral mucosal epithelial cell sheets. We developed a cell transportation technique for clinical trial to bridge hospitals.

Methods: A cell transport container that can maintain and monitor the inside temperature and air pressure was developed. Human oral mucosal epithelial cells obtained from two healthy volunteers were cultured on temperature-responsive culture dishes. Epithelial cell sheets were transported between Osaka University and Tohoku University using the cell transport case by airplane. The cell sheets were evaluated before and 12 hours after transportation. Histological and immunohistochemical analyses were performed for cell sheets. Cell viability and purity of cell sheets were evaluated by flow cytometry.

Results: Temperature inside the case was kept above 32°, and the change of air pressure was within 10 hPa during transportation. Cell sheets were well stratified and harvested successfully after transportation. The expression patterns of keratin 3/76, p63, ZO-1 and MUC16 were equivalent before and after transport. The cell viability was 72.0% before transport and 77.3% after transport. Epithelial purity was 94.6% before transport and 87.9% after transport.

Conclusion: Oral mucosal epithelial cell sheets can be transported using this new transport system with quality maintenance. We are conducting multicenter clinical trial using this transportation technique.

IRB Status: Approved

Disclosures:

YOSHINOBU OIE, MD, PHD: Lecture fee relationship with Otsuka, Santen and Alcon

O181

MATURATION OF HUMAN CORNEAL ENDOTHELIAL CELLS CULTURED ON SELF-ASSEMBLED FIBROBLAST MATRIX

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SCHEPENS EYE RESEARCH INSTITUTE, MEE DEPARTMENT OF OPHTHALMOLOGY, HARVARD MEDICAL SCHOOL

We examined if a cell-based 3-dimensional (3D) corneal construct consisting of human corneal fibroblasts (HCF) stimulated by a stable vitamin C derivative (VitC) to self-assemble an extracellular matrix served as a good substrate to examine corneal endothelial maturation and basement membrane assembly. HCF were grown on Transwells in EMEM with 10% FBS and 0.5mM VitC for 4 weeks, and endothelial cell line (HCEn), was cultivated on the constructs for another 5-14 days. Cultures were examined by light and transmission electron microscopy (TEM), indirect-immunofluorescence microscopy (IF: ZO-1, ALDH1A1, Laminin, Ki67 and Caspase-3), and gelatin zymography. TEM revealed that the HCEn developed a monolayer on the construct and formed well-defined cell-cell junctions, which was confirmed with the localization of ZO-1. Both ALDH1A1 and laminin were present in the endothelial cells, and laminin also was localized in the interface between the HCEn and the fibroblast matrix. In addition, minimal numbers of HCEn cells were labeled with Ki67 or Caspase-3. Zymography indicated that the addition of HCEn to the construct stimulated the secretion of a protein band corresponding to MMP9. In the current investigation, we demonstrated that our 3D construct is a good substrate for HCEn, which attached, grew, and matured on the matrix. They also formed a monolayer, expressed markers for maturation (ZO-1 and ALDH1A1), and shared the in vivo characteristics of minimal proliferative and apoptotic activity. In addition, HCEn appeared to express and secrete laminin, suggesting that the combination of HCEn on the construct could provide a model for the examination of Descemet's membrane formation. Finally, the combination of the two cell types appears to stimulate secretion of MMPs, which may allow for examination of wound healing-like processes.

IRB Status: International

Disclosures:

JAMES ZIESKE, PHD: No financial relationships to disclose

O182

MICRO RNAs EXPRESSED IN NORMAL AND DIABETIC HUMAN CORNEAS

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CEDARS-SINAI MEDICAL CENTER, BIOMEDICAL SCIENCES¹; UCLA²; CEDARS-SINAI MEDICAL CENTER, GENOMICS CORE³

MicroRNAs (miRNAs) post-transcriptionally regulate gene expression in many tissues and affect a wide range of biological processes, but their roles in normal and diseased limbal epithelial stem cells (LESC) remain unknown. Our purpose was to identify and characterize miRNAs expressed in the central and limbal compartments of human normal and diabetic corneas, and to examine their roles in cell migration, proliferation and differentiation. Deep sequencing was used to quantitatively identify differential expression of miRNAs in these regions. Using supervised analysis, we identified several miRNAs upregulated (>2 cutoff; $p < 0.05$) in limbus vs. central corneas such as miR-145-5p, -136-3p and 146a-5p. Quantitative RT-PCR and *in situ* hybridization corroborated our findings. Some miRNAs such as 146a were more expressed in the LESC-harboring basal cell layer suggesting their roles in stem cell maintenance. MiR-146a was also upregulated in both central and limbal diabetic vs. normal cornea. Overexpression of miR-146a in LESC led to slow wound closure compared to the cells transfected with scrambled controls. Respective inhibitor (antagomir) significantly enhanced wound healing and led to an increase in the expression of migration- and survival-related signaling molecules, p-EGFR, and p-p38 vs. negative controls. Furthermore, organ-cultured human diabetic corneas transfected with miR-146a inhibitor displayed normalization of wound healing rate in comparison to the fellow corneas transfected with scrambled control. These data suggest that miR-146a may be involved in the maintenance of undifferentiated LESC, and its expression is downregulated during their radial migration towards the central cornea and completion of terminal differentiation. Furthermore, miR-146a upregulation may be an important mechanism of diabetic alterations in corneal wound healing. Therefore, miRNA gene therapy could be potentially useful for treating corneal diseases with abnormal wound healing and/or stem cell dysfunction.

IRB Status: None

Disclosures:

MEHRNOOSH SAGHIZADEH, PHD: No financial relationships to disclose

CO10 – Somatic and Pluripotent Stem Cell Technology: Toward the Corneal Regeneration

O183

GENE AND CELL THERAPY APPROACHES FOR CORNEAL EPITHELIAL DISORDERS

ALEXANDER LJUBIMOV, Andrei Kramerov, Michael Winkler, Tanya Spektor, Mehrnoosh Saghizadeh

CEDARS-SINAI MEDICAL CENTER, REGENERATIVE MEDICINE INSTITUTE

Certain vision-threatening corneal diseases including dystrophies and limbal stem cell deficiency (LSCD) are related to dysfunction and/or degeneration of corneal epithelial cells and their progenitors. In recent years, it became possible to normalize diseased corneal epithelium with gene and stem cell therapy. Gene therapy has been attempted in model

systems and in animals using plasmids, viruses or nanoparticles. Successful delivery of *bcl-xL* gene via eye drops reduced keratocyte apoptosis upon epithelial debridement. *Glial-derived neurotrophic factor* gene therapy enhanced survival of corneal epithelium in culture and in bioengineered corneal constructs. Delivery of antisense to opioid growth factor receptor accelerated epithelial wound healing in rat corneas. We were able to correct diabetic corneal wound healing abnormalities using gene therapy. A combination therapy with overexpression of *c-met* proto-oncogene and shRNA silencing of *cathepsin F* and *matrix metalloproteinase-10* genes was found to be significantly more effective in restoring normal epithelial wound healing than any monotherapy. This treatment also resulted in normalization of putative stem cell marker expression in diabetic organ-cultured corneas. microRNAs are emerging as new powerful tools for gene therapy. Our data show that some microRNAs can accelerate corneal epithelial wound healing. Successful use of limbal epithelial cell therapy (limbo-keratoplasty) was reported in granular, lattice, and gelatinous drop-like corneal dystrophies. Cultured limbal epithelial stem cells have been used to successfully treat hundreds of patients with LSCD in India, U.K., Italy, and Germany. Alternative sources of these cells for bilateral LSCD are being developed including oral mucosa, hair follicle, and conjunctiva, as well as their xenobiotic-free cultures. Recent progress in programming embryonic and induced pluripotent stem cells into corneal epithelium including our data offers new possibilities for creating renewable and bankable sources of corneal epithelial stem cells for restoring vision in patients with LSCD and other epithelial diseases of the cornea.

IRB Status: Approved

Disclosures:

ALEXANDER LJUBIMOV, PHD, DSC: No financial relationships to disclose

O184

NOVEL APPROACHES TO BIOENGINEERING LIMBAL STEM/PROGENITOR CELLS

SOPHIE DENG

JULES STEIN EYE INSTITUTE, UCLA

A minimal number of limbal stem/progenitor cells (LSCs) is required to achieve a successful clinical outcome to treat LSC deficiency. The goal of ex vivo expansion of limbal epithelial cells is to expand the stem/progenitor population and mouse 3T3 cells are the most efficient feeder cells to support the growth of human LSCs. A novel 3-dimensional (3D) culture was developed to allow for a close contact with the feeder cells while maintaining the polarity of the LSCs. The expansion of LSCs on these human feeder cells had a similar efficiency as on the 3T3 feeder cells only when LSCs were cultured in the form of cell clusters. Therefore, maintaining the cell-cell contact and polarity of LSCs in culture appears to be more important to preserve the stem/progenitor phenotype than the type of feeder cells. In addition, Wnt signaling regulates LSCs proliferation. By modulating the Wnt signaling in culture, the efficiency of LSC growth could be enhanced. These new bioengineering

approaches could increase the efficiency of ex vivo expansion of the stem/progenitor cells for transplantation.

IRB Status: Approved

Disclosures:

SOPHIE DENG, MD, PHD: No financial relationships to disclose

O185

INDUCED PLURIPOTENT STEM CELL TECHNOLOGY FOR THE TREATMENT OF CORNEAL ENDOTHELIAL DISEASE

SHIGETO SHIMMURA

OPHTHALMOLOGY, KEIO UNIVERSITY SCHOOL OF MEDICINE

Treating corneal disease with somatic or pluripotent stem cells is becoming a reality due to advances in stem cell biology and tissue engineering techniques. However, there are still several issues that require attention including safety and regulatory matters before clinical application can be achieved. Steps towards realizing pluripotent stem cell therapy include a proof-of-concept approach using tissue specific stem/progenitor cells, establishment of surgical techniques and deriving target cells from pluripotent stem cells. Since the anterior chamber is an immunologically privileged site, developing cell therapies from allogeneic pluripotent stem cells (iPSCs) is less of a challenge compared to other organs. Producing large quantity of iPSC-derived endothelium from a safety-certified iPS clone may reduce therapeutic cost, while solving the problem of donor shortage worldwide. We have previously reported the isolation of neural crest stem cells from the cornea stroma. These cells were then engineered to become an endothelial phenotype with full NaK-ATPase function and tight junction formation. We then developed a protocol to induce neural crest cells from monkey and human iPSCs, which can then be engineered to corneal endothelium-like cells (iCEC). iCECs express major corneal endothelial markers such as ATP1A1, K8, COL8A2 and CDH2. iCEC can also rescue a bullous keratopathy model in rabbits, suggesting that these cells have sufficient pump function. Further refinement in culture protocol will lead to way to clinical trials in humans.

IRB Status: Approved

Disclosures:

SHIGETO SHIMMURA, MD: Patents/Royalties relationship with Keio University

O186

CELL BASED APPROACH FOR THE TREATMENT OF CORNEAL ENDOTHELIAL DYSFUNCTION

NORIKO KOIZUMI

DOSHISHA UNIVERSITY

The concept of our new surgical treatment for corneal endothelial dysfunction involves replacing the damaged cell layers with healthy corneal endothelial cells (CECs) cultivated and proliferated in vitro. Previously, we reported the feasibility of

cultivated CEC sheet transplantation using a type I collagen carrier in a monkey corneal endothelial defect model in which the proliferative ability of CECs are severely limited, like as in humans. In 2009, we reported that the selective Rho-associated kinase (ROCK) inhibitor Y-27632 promotes cell adhesion and proliferation and inhibits the apoptosis of primate corneal endothelial cells in culture. Currently, we are developing a cell-injection therapy using cultivated CECs in combination with ROCK inhibitor. We confirmed the efficacy and safety of the cell-injection therapy using both rabbit and monkey models, and we are now moving onto its clinical application. In regard to that clinical application, several problems associated with human CEC culture need to be resolved. The in vitro expansion of human CECs is still very difficult due to the fact that they do not proliferate well, and even when they do proliferate, they easily transform into a fibroblastic phenotype. Recently, we established a cultivation protocol of human CECs for clinical application which includes the use of ROCK inhibitor, conditioned medium obtained from mesenchymal stem cells, and the inhibition of transforming growth factor beta signaling. The protocol for the current clinical trial was approved by the Japanese Ministry of Health, Labor and Welfare in March 2013 and the first-in-man clinical study was recently started at Kyoto Prefectural University of Medicine, Kyoto, Japan.

IRB Status: Approved

Disclosures:

NORIKO KOIZUMI, MD, PHD: Patents/Royalties relationship with Senju Pharmaceutical Co., Ltd.; Patents/Royalties relationship with JCR Pharmaceuticals Co., Ltd.

O187

CORNEAL EPITHELIAL STEM CELLS AND NICHE MICROENVIRONMENTS

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UNIVERSITY OF ERLANGEN-NÜRNBERG, DEPARTMENT OF OPHTHALMOLOGY

Maintenance and regeneration of the corneal epithelium relies on unipotent progenitor cells at the corneoscleral limbus. It is assumed that maintenance of limbal progenitors is governed by a number of extrinsic factors. These are provided in vivo by the local microenvironment, which has been termed the "stem cell niche." The postulated limbal niche is an anatomically protected site of intimate epithelial-stromal communication and is highly vascularised, innervated, pigmented due to melanocytes, infiltrated with immune cells, and supported by a specialized extracellular matrix as well as different populations of mesenchymal cells emitting soluble signals. This lecture outlines our current understanding of the structural components of the limbal niche including specific extracellular matrix components, cell-matrix- and cell-cell adhesion molecules, and various niche cell populations, which are involved in stem cell regulation through multiple signaling pathways including the Wnt/ β -catenin, Notch, BMP/TGF- β , and Hedgehog pathways. Depletion of the stem cell reservoir and/or destruction of its niche microenvironment can cause severe ocular surface disease. For ex vivo expansion and transplantation of limbal epithelial cells, limbal

progenitors are removed from their niche. To maintain their "stemness," niche factors need to be replicated by the culture system. For that purpose, biochemically modified hydrogels engineered to incorporate specific niche components represent a promising strategy to generate biomimetic constructs that may regulate stem cell function in vitro.

IRB Status: International

Disclosures:

URSULA SCHLÖTZER-SCHREHARDT, PHD: No financial relationships to disclose

O188

STEM CELL AND CORNEAL REGENERATIVE MEDICINE

KOHI NISHIDA

OSAKA UNIVERSITY GRADUATE SCHOOL OF MEDICINE

Corneal epithelial stem cells are known to be localized to the basal layer of the limbal epithelium. This corneal stem cell concept has been first reported in 1980s, based on the

findings that label-retaining cells are located in the limbal basal epithelium. Since then, several investigators reported the specific characteristics for corneal epithelial stem/progenitor cells, including high colony-forming potential, p63 positive and so on. We have recently demonstrated that corneal epithelial stem/progenitor cells can be enriched in integrin $\alpha 6 \beta 1$ /CD71dim fraction by FACS.

Complete loss of corneal epithelial stem cells because of severe trauma eye disease leads to corneal vascularization and opacification with severe visual loss. For corneal reconstruction in patients with such limbal stem cell deficiencies, we previously developed a unique method using tissue-engineered epithelial cell sheets comprising only the patient's autologous oral mucosal epithelium. We are currently studying the potential of pluripotent stem cells for the treatment of corneal diseases. In this presentation, I will talk about the recent progress of stem cell therapy for corneal diseases.

IRB Status: Approved

Disclosures:

KOHI NISHIDA, MD, PHD: Lecture fee relationship with HOYA, AMO, Otsuka, Santen, Johnson & Johnson, Pfizer, Alcon, Takeda, Wacamoto and Novartis

Ocular Immunology

IM01 – Autoimmunity and Immune Privilege

O189

COMMENSAL MICROBIOTA AS A TRIGGER OF AUTOIMMUNE UVEITIS

RACHEL CASPI¹, Reiko Horai¹, Carlos Zárate-Bladés¹, Jun Chen¹, Phyllis Silver¹, Patricia Dillenburg-Pilla², Yingyos Jittayasothorn², Chi-Chao Chan², Hidehiro Yamane³, Kenya Honda⁴

LABORATORY OF IMMUNOLOGY, NEI, NIH¹; NIH, OPCB, NIDCR²; NIH, LI, NIAID³; RIKEN, YOKOHAMA, JAPAN⁴

Autoimmune uveitis is believed to be driven by autoreactive T cells, but the stimuli that activate them are unknown. Microbial triggers in human uveitis have been suspected, but never proven. We studied this question in a new mouse model of spontaneous uveitis. R161H mice express a transgenic retina-specific T cell receptor and develop 100% spontaneous uveitis by 2–3 months of age. Retina-specific Th1 and Th17 cells infiltrate their eyes and are also present in the gut lamina propria (LP). Treatment with antibiotics, or germ-free conditions result in a strongly attenuated uveitis, with fewer Th17 cells in the gut LP. Interestingly, R161H T cells appeared to be activated more strongly in the gut than polyclonal T cells by several criteria: (a) conventionally housed R161H mice consistently displayed 2–5x more Th17 cells in their LP compared to WT littermates, even when crossed onto an IRBP-deficient background; (b) *in vivo*, R161H adopted the Th17 phenotype in the gut with higher frequency and (c) compared to polyclonal T cells, R161H TCR transgenic T cells were more readily activated by fecal extracts *in vitro*. This suggested involvement of the IRBP-specific TCR in their activation. Experiments designed to test that, utilizing Nur77 reporter mice and staining for pZap70, revealed clear evidence that the TCR of R161H cells is being engaged in the gut environment. We propose that commensal microflora can activate circulating retina-specific T cells to cause spontaneous autoimmune uveitis, at least in part through a process dependent on TCR signaling (mimicry?). These data not only have implications for etiology of human uveitis, but also suggest that activation of autoreactive TCRs by commensal microflora may be a more common trigger of autoimmune diseases than is currently appreciated.

IRB Status: Approved

Disclosures:

RACHEL CASPI, PHD: No financial relationships to disclose

O190

ANALYSIS OF RETINAL ANTIGEN-SPECIFIC TREGS AND DENDRITIC CELLS IN RETINAL T CELL RESPONSES AND EAU

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UNIVERSITY OF MINNESOTA, OPHTHALMOLOGY AND VISUAL NEUROSCIENCES

We previously reported that peripheral Tregs (pTregs) generated “on-demand” in retina were key components of retinal immune privilege, and that retinal dendritic cells (DC) provided APC activity that promoted development of Tregs and Teffs from naive antigen-specific T cells. These observations were expanded by examining the role of locally generated, locally acting pTregs in spontaneous EAU, and testing the role of local DC in antigen presentation in retina. Several transgenic (Tg) mice were used including: arrbgal mice expressing beta-galactosidase as a retinal neo-self antigen; TCR Tg mice (BG2) specific for bgal; Foxp3-GFP mice with GFP-labeled Tregs; Foxp3-DTR/GFP mice with GFP-labeled and DTx-depletable Tregs; CD11c-DTR/GFP mice expressing the DTR and GFP on DC; and several combinations of these mice derived from backcrossing onto arrbgal mice and BG2 mice to give a variety of mice with bgal-specific T cells in combination with the other transgenes. Treg and DC depletion from retina was done by DTx injection into the eye. T cells and DC were assayed by FACS of retina or blood. Retinal autoimmunity was assessed by histological analysis. Local antigen presentation was tested by inoculation of bgal into the anterior chamber and analyzing retina by FACS. After bgal (FDG/bgalmice) or IRBP (FDG mice) immunization, Treg depleted retinas showed increased incidence and severity of autoimmunity. We found local depletion of Tregs from retina sufficient to induce spontaneous autoimmunity in FDG/bgalmice but not in single or double Tg mice. DC depletion from the retina prevented Treg and Teff generation within retina after bgal injection. Microglia remaining after DC depletion did not make up for loss of DC-dependent antigen presentation. We conclude that local retinal Tregs protect against spontaneous organ-specific autoimmunity, and local retinal DC are necessary for antigen presentation to T cells.

IRB Status: None

Disclosures:

DALE GREGERSON, PHD: No financial relationships to disclose

O191

AUTOIMMUNE MECHANISMS IN AGE RELATED MACULAR DEGENERATION

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The exact molecular etiology of age-related macular degeneration (AMD) remains unknown and represents a major impediment in developing useful therapies. Factors such as inflammation and oxidative stress have been identified as

contributing factors. Elevated levels of a lipid peroxidation product, CEP, and elevated anti-CEP autoantibodies have been correlated with AMD disease state. This data along with other emerging data suggests a role for the immune system and specifically autoimmunity in AMD pathogenesis. Work from our lab has demonstrated CEP as the molecular link between oxidative stress and inflammation, such that immunization of mice with CEP-adducts leads to AMD-like lesions, anti-CEP antibody production, and retinal macrophage recruitment. However, the specific roles of the adaptive immune system and innate immune system have not been studied in detail. In the CEP-immunized mouse model of AMD, we found M1 polarized macrophage retinal infiltration. Additionally, we identified IFN- γ producing CEP-specific T cells indicating a Th1 response. In vitro, CEP-specific T-cells promote M1 macrophage polarization in co-culture experiments. We also found that B cells are dispensable in our AMD model, since B-cell deficient mice still develop retinal pathology. Finally, we show that T-cell immunosuppressive therapy actually inhibited CEP-mediated pathology, pointing to a role for T-cells in AMD development. CEP not only functions to coordinate an adaptive immune response, but also impacts innate immune cell function. In *in vitro* macrophage cultures, CEP-adducts induce inflammatory signaling. Specifically, we found upregulation of inflammatory cytokines such as IL-12, TNF- α , and IL-1 β , indicating M1 macrophage polarization, which mirrors our *in vivo* findings of retinal infiltrating M1 macrophages in CEP-immunized mice. Overall, our findings suggest that CEP triggers an inflammatory response and may play an initiating role in retinal inflammation that leads to AMD. This data provides new insights into the pathogenesis and possible treatment of AMD.

IRB Status: None

Disclosures:

VICTOR PEREZ, MD: Patents/Royalties relationship with SKS Ocular; Consultant/Advisor relationship with Allergan; Consultant/Advisor relationship with Eleven; Consultant/Advisor relationship with Bausch & Lomb; Consultant/Advisor relationship with Rigel

O192

CORNEAL CELL-MEDIATED IMMUNE REGULATION AND IMMUNE PRIVILEGE

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NIPPON MEDICAL SCHOOL, DEPARTMENT OF OPHTHALMOLOGY

The eye, which is endowed with immune privilege, is a rare organ that permits analysis of the regulatory mechanisms for inflammation in organs. Ocular tissue modifies and regulates immune responses for preventing inflammation-mediated tissue destruction, by using differently functioning molecules. In particular, studies using animal models of corneal transplantation have revealed the molecular mechanisms of corneal cell-mediated immune privilege. Several molecules expressed in the cornea induce apoptosis of T cells and delete effector T cells in the cornea. Constitutive expression of Fas ligand (FasL, CD95L) and programmed death ligand 1 (PD-L1, B7-H1) on corneal endothelial cells induce apoptosis of effector T cells via

Fas and PD-1, respectively. PD-1 on the surface of the T cells is up-regulated as a result of contact with the corneal cells, thus accelerating apoptosis. B7-H3 is also constitutively expressed in the corneal endothelium and iris ciliary body and plays a role in the induction of eye-derived immune tolerance so called anterior chamber associated immune deviation (ACAID). We have also found that constitutive expression of glucocorticoid-induced TNF receptor family-related protein ligand (GITRL) in the cornea mediates local expansion of CD25+CD4+T regulatory cells and suppresses conventional effector T cell function via GITR. Thus, these differently functioning molecules contribute to maintenance of the local immune suppressive microenvironment in the cornea. The current study for the roles of B7RP-1 and Galectine-9 expressed in the cornea are also introduced.

IRB Status: None

Disclosures:

JUNKO HORI, MD, PHD: No financial relationships to disclose

O193

RESTORING IMMUNOSUPPRESSIVE FUNCTION OF REGULATORY T CELLS REDUCES CORNEAL INFLAMMATION IN HIGH RISK CORNEAL TRANSPLANTATION

MARYAM TAHVILDARI, Masahiro Omoto, Yihe Chen, Sunil Chauhan, Reza Dana

SCHEPENS EYE RESEARCH INSTITUTE, MASSACHUSETTS EYE AND EAR, DEPARTMENT OF OPHTHALMOLOGY, HARVARD MEDICAL SCHOOL

It is suggested that decreased immunosuppressive function of regulatory T cells (Treg) can contribute to corneal allograft rejection. Using a mouse model of corneal transplantation, we evaluated graft leukocyte infiltration in high risk (HR) corneal allografts after treatment with low dose interleukin-2 (IL-2), a cytokine known for its role in enhancing Treg function. BALB/c (H-2d) mice were used as recipients and C57BL/6 (H-2b) mice served as donors. Intrastromal sutures were placed in the host corneas 2 weeks prior to transplantation to create a HR bed. Graft recipients received 3 intraperitoneal injections (once a day) of IL-2 (1 μ g/20g body weight in 100 μ l of saline) before transplantation; saline injected mice with either inflamed (HR) or uninflamed low risk (LR) corneas were used as controls. Injections were continued twice/week until 2 weeks post-transplantation when corneas and draining lymph nodes (DLN) were harvested. Flow cytometry analysis of corneal allografts demonstrated significantly decreased frequencies of CD45+ cells (leukocytes) in the HR IL-2 treated group (7.9%) compared to either HR controls (13.1%, $p=0.036$) or LR controls (11.04%, $p=0.005$). CD4+ T cells constituted 1.4% of graft cells in the HR IL-2-treated group vs. HR controls (2.7%, $p=0.003$) and LR controls (1.8%, $p=0.045$). In DLNs, flow cytometry showed increased frequencies of CD4+CD25+Foxp3+ Tregs in the HR IL-2-treated hosts (14.91% \pm 0.4) compared to both HR (13.42% \pm 0.5) and LR (14.47% \pm 0.2) controls. CTLA-4 expression levels (as measured by mean fluorescent intensity) was increased among Foxp3+Tregs in the HR IL-2 treated group (225 \pm 2.6) vs. HR controls (206 \pm 5.2) and LR controls (210 \pm 6.5), demonstrating

enhanced Treg suppressive function. In conclusion, low dose IL-2 treatment reduces corneal inflammation in HR corneal transplantation below the levels observed in LR transplants, while restoring Treg numbers and their function. These findings suggest that Treg targeted therapies may decrease corneal rejection rates in high risk corneal transplantation.

IRB Status: None

Disclosures:

MARYAM TAHVILDARI, MD: No financial relationships to disclose

IM02 – Inflammatory Mediators in Age-Related Macular Degeneration

O194

MICROGLIA/MACROPHAGE BEHAVIOR DURING AGE-RELATED MACULAR DEGENERATION

ANDREW DICK

BRISTOL EYE HOSPITAL

Myeloid cells are highly adaptable to environmental cues that regulate tissue responses to injury, inflammation or degeneration. The retina and choroid remain healthy as a result of keeping in check the activation status of myeloid cells (microglia and choroidal macrophages) via, for example CD200R and TREM-2. Following inflammation, as observed in Experimental Autoimmune Uveoretinitis (EAU), the tissue myeloid cell number and activation do not return to premorbid state and the result, even in absence of overt clinical inflammation is continued tissue damage and neovascularisation – altered health. Resetting the threshold of activation of myeloid cells is readily observed in the laser induced angiogenesis model (L-CNV), where angiogenic response is precipitated by early influx of CCR2 monocytes as well as microglia to site of laser injury. This can be attenuated and perturbed via augmenting CD200R signaling or via alternative activation of cells via IL-4 or 13 conditioning. The mechanisms will be discussed but the myeloid cell restricted responses affords opportunities to alter the retinal environment, reset myeloid activation responses and return tissue health.

IRB Status: None

Disclosures:

ANDREW A. DICK, BSC (HONS): Consultant/Advisor relationship with Novartis; Lecture Fees relationship with ABBvie

O195

THE ALTERNATIVE PATHWAY OF COMPLEMENT IN AGE-RELATED MACULAR DEGENERATION

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Age-related macular degeneration (AMD) is a slowly progressing multifactorial disease involving genetic abnormalities and

environmental insults. Genetic studies have demonstrated that polymorphisms in different complement proteins each increase the risk for developing AMD, and oxidative stress is a known environmental risk factor. As the alternative pathway is continuously activated in the fluid phase, tissue surfaces require continuous complement inhibition to prevent spontaneous autologous tissue injury. To explore the hypothesis that AMD pathology results from uncontrolled activation of the alternative pathway (AP) of complement, we have utilized stable RPE monolayers challenged with oxidative stress (H₂O₂ or cigarette smoke extract) and complement-sufficient serum to mimic oxidative stress in the presence of complement proteins. We have previously shown that oxidative stress down-regulates the level of complement inhibition at the cell surface of RPE cells, effectively reducing the cell's ability to fight off complement attack. Here we will provide an update on how complement attack, which is sublytic and transient in nature, results in the secretion of cytokines and matrix-metalloproteinases, endoplasmic reticulum stress and lipid droplet formation, generating a microenvironment conducive to AMD-related pathologies. Using a combination of complement-depletion and reconstitution strategies, we have shown that the lectin pathway is required to initiate the complement cascade, which is then further amplified by the AP. Finally, we provide evidence that endogenous complement factor H cannot inhibit the AP in oxidatively-stressed cells as it is unable to bind to required ligands on the cell surface, while an CFH-based inhibitor that utilizes an alternative membrane targeting strategy is effective in providing protection. Although we can only extrapolate from cell-based models to humans, identifying how sublytic complement activation interferes with normal RPE function might offer opportunities for future therapeutic interventions in ocular diseases involving complement dysregulation.

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IRB Status: Verified

Disclosures:

BAERBEL ROHRER, PHD: Patents/Royalties relationship with Alexion

O196

ACTIVATION OF INNATE IMMUNE SYSTEM VIA PATTERN RECOGNITION RECEPTORS IN AGE-RELATED MACULAR DEGENERATION

KAI KAARNIRANTA

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Age-related macular degeneration (AMD) is the most common cause of irreversible loss of colour and sharp vision. Cell cultures, histopathological stainings and blood sample analysis have demonstrated that inflammation is the key player in the pathogenesis of AMD. Genetic studies have revealed that complement cascade strongly associates with the development of AMD. However, innate immunity defence involves several other pattern recognition receptors (PRRs) which can trigger

inflammatory responses. Retinal pigment epithelial (RPE) cells have the main role in the immune defence in macula. The endogenous danger signals which can activate different PRRs in RPE cells, such as Toll-like, NOD-like and scavenger receptors along with complement system. Chronic oxidative stress, lipid peroxidation and lysosomal lipofuscin formation are well-known danger signals to RPE cells. Hallmarks of AMD are accumulation of lipofuscin and extracellular drusen that also indicate impaired proteolysis in RPE cells. Thus AMD is also aggregation disease. During aging impaired proteosomal and lysosomal proteolysis lead to both intra- and extracellular protein aggregation and innate immune response in RPE cells that coincides with the severity level of AMD.

IRB Status: None

Disclosures:

KAI KAARNIRANTA, MD, PHD, MSC: No financial relationships to disclose

O197

DYSREGULATED LYSOSOMAL-MEDIATED CLEARANCE SHIFTS PARA-INFLAMMATION TO CHRONIC INFLAMMATION IN RPE CELLS

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JOHNS HOPKINS UNIVERSITY, OPHTHALMOLOGY¹; NATIONAL EYE INSTITUTE²

Several studies support the hypothesis that the immune system is involved in the pathogenesis of age-related macular degeneration (AMD), in concert with, or in addition to, other factors. While chronic inflammation is believed to contribute to the pathology of AMD, knowledge regarding the events that elicit the change from para-inflammation to chronic inflammation in the pathogenesis of AMD is lacking. Para-inflammation is intermediate between the basal homeostatic state and a chronic inflammatory response. Severe or prolonged impairment of lysosomal-mediated clearance in the retinal pigment epithelium (RPE) cells may convert para-inflammation to chronic inflammation, leading to retinal degeneration. We have recently shown in the Nuc1 rat (a spontaneous mutation in the *Cryba1* gene encoding for β A3/A1-crystallin) and in mice lacking β A3/A1-crystallin specifically in the RPE, that impaired lysosomal clearance decreases both autophagy and phagocytosis. This leads to the accumulation of lipofuscin within RPE cells, subretinal lesions in the posterior pole, and deposits between the RPE and Bruch's membrane. We now report that lipocalin-2 (LCN2), a mammalian innate immunity protein that is trafficked to the lysosomes, accumulates in the RPE cells of *Cryba1* conditional knockout (cKO) mice. LCN2 has been shown to be markedly elevated in serum and tissues during inflammation. Since, in macrophages, LCN2 is initially endocytosed into early endosomes and subsequently sorted to lysosomes, one would expect it to accumulate in *Cryba1* cKO RPE with age, since lysosomal-mediated clearance is impaired in these mice. Levels of LCN2 increase with age in the cKO mice, accompanied by increases in chemokine (C-C

motif) ligand 2 (CCL2), reactive gliosis, and immune cell infiltration. Here, we link LCN2 and its induced chronic inflammatory responses with pathological changes resembling AMD in a genetic mouse model.

IRB Status: None

Disclosures:

DEBASISH SINHA, PHD: No financial relationships to disclose

O198

CIGARETTE SMOKE (CS), CYTOPROTECTIVE NRF2 SIGNALING AND THE INNATE IMMUNE RESPONSE DURING AGE-RELATED MACULAR DEGENERATION (AMD)

JAMES HANDA, Marisol Cano, Lei Wang, Katayoon Ebrahimi, Hong Wei

WILMER EYE INSTITUTE, JOHNS HOPKINS UNIVERSITY

CS is the strongest environmental risk factor for AMD, yet we don't understand its pathophysiologic contribution. RPE cells exposed to CS extract (CSE) respond with a robust Nrf2 mediated antioxidant response that includes resident mitochondrial antioxidant genes along with a protective unfolded protein response (UPR). At higher CSE, we found a lack of transcriptional induction of Trx2, Prx3, and SOD2, which was associated with reduced ATP and mitochondrial superoxide generation. CSE injected intravitreally into C57Bl6J mouse eyes produced a similar protective UPR with decreased ATP and mitochondrial TOM20 abundance, along with evidence of RPE epithelial-mesenchymal transition. At the same time, RPE cells stimulated with CSE had increased complement C3a and C3b, but not iC3b, along with a reduction in complement regulators CD46, CD55, and CD59. C3a generation led to IL-1b induction that was mediated through C3aR while C3b led to sublytic C5b-9 complex formation. Nrf2-knockdown impaired Nrf2 mediated cytoplasmic and mitochondrial antioxidant gene expression with CSE, which led to enhanced protein carbonylation, reduced glutathione, and a reduction in the threshold of cell death from 500ug/ml to 250ug/ml CSE. While Nrf2-KD alone induced the UPR, the response was not magnified with the addition of CSE, while the decreased ATP induced by CSE was magnified by Nrf2-KD. Mitochondrial dysfunction was severe enough with Nrf2-KD after 500ug/ml CSE that cytochrome c was released into the cytoplasm along with caspase 3 cleavage. In C57Bl6J mice exposed to CS for 6 months, RPE Nrf2 signaling decreased along with increased 8OHdG immunolabeling. This pro-oxidative stress environment increased C3, C3a, and IL-1b. Collectively, these data suggest that RPE cells with Nrf2 impairment have magnified mitochondrial dysfunction after CS exposure, which can lead to activation of the intrinsic apoptosis pathway. Simultaneously, CS induces a very specific pro-inflammatory C3 complement response that is magnified by Nrf2 deficiency.

IRB Status: None

Disclosures:

JAMES T. HANDA, MD: No financial relationships to disclose

IM03 – New Insights into the Immunology of Ocular Tumors

O199

MACROPHAGE INFILTRATION IN UVEAL MELANOMA: ENVIRONMENT OR GENETICS?

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Many parameters are associated with a bad prognosis in uveal melanoma, such as a large tumor size, chromosome 3 loss, certain RNA patterns, or the presence of an inflammatory phenotype. An inflammatory phenotype includes lymphocytes and macrophages. As VEGF is an attractant for macrophages, we considered the possibility that rapid tumor growth might lead to hypoxia and VEGF release by tumor cells, which would bring in macrophages. We tested this possibility in vitro.

Uveal melanoma cells were cultured under normoxic and hypoxic conditions and the cell culture supernatant was tested for its capacity to affect monocyte migration or differentiation. We also determined the presence of chemotaxis-associated cytokines in the supernatant by ELISA and in cells by PCR.

Exposure of freshly-cultured uveal melanoma cells to hypoxia led to an increase in the production of pro-inflammatory cytokines such as VEGF, PlGF, TGFβ. The supernatant of some uveal melanoma cell cultures was able to induce chemotaxis, but this was independent of hypoxia. UM supernatant inhibited monocyte differentiation.

We can conclude that hypoxia stimulates the production of a wide range of cytokines in uveal melanoma. Cultured UM cells also produce substances that stimulate monocyte chemotaxis. However, the production of such cytokines was independent of hypoxia, and varied between cultures. The finding that UM cell supernatant inhibited monocyte differentiation may be an indicator that UM cells may also affect other antigen-presenting cells.

IRB Status: International

Disclosures:

MARTINE JAGER, MD, PHD: No financial relationships to disclose

O200

TUMOR-ASSOCIATED LYMPHANGIOGENESIS AND ITS INHIBITION IN OCULAR SURFACE MALIGNANCIES

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The human conjunctiva is physiologically well-endowed with blood and lymphatic vessels. The induction of angiogenesis

with sprouting of new capillary blood vessels from preexisting ones is generally considered as one of the six hallmarks of cancer, since tumors require sustenance in the form of nutrients and oxygen as well as an ability to evacuate metabolic wastes and carbon dioxide. Furthermore, the outgrowth of new from preexisting lymphatic vessels, the so-called tumor-associated lymphangiogenesis, is regarded as the initial step in lymphogenic metastasis of several non-ocular malignant tumors. Recently, tumor-associated lymphangiogenesis could be detected in malignant tumors affecting the ocular surface (conjunctival melanomas, conjunctival carcinomas, ciliary body melanomas with extraocular extension), and associated with prognostic significance for the risk of local recurrence, lymphatic spread, distant metastasis and tumor-related death. In the future, novel antihemangiogenic and antilymphangiogenic therapies might help to enhance survival of these ocular tumor patients.

IRB Status: Approved

Disclosures:

LUDWIG HEINDL, MD, PHD: No financial relationships to disclose

O201

AUTOCRINE IMPACT OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) A ON PRIMARY AND METASTATIC UVEAL MELANOMA CELLS

KONRAD KOCH¹, Nasrin Refaian¹, Deniz Hos¹, Simona Schlereth¹, Jacobus Bosch², Claus Cursiefen¹, Ludwig Heindl¹

DEPARTMENT OF OPHTHALMOLOGY, UNIVERSITY OF COLOGNE¹; DEPARTMENT OF INTERNAL MEDICINE 5, HEMATOLOGY AND ONCOLOGY, UNIVERSITY OF ERLANGEN-NUREMBERG²

Tumor-derived vascular endothelial growth factor A (VEGF-A), apart from expediting sufficient vascularization, subsequent tumor growth and metastatic spread, can act on malignant cells themselves provided that VEGF receptors 1 or 2 (VEGF-R1, -R2) are co-expressed. The study goal was to investigate whether such autocrine VEGF-A signaling exists in uveal melanoma (UM). Primary (MEL-270, OM-431) and metastatic (OMM-2.3, OMM-2.5) UM cells were analyzed for VEGF-A, VEGF-R1, and VEGF-R2 expression by RT-PCR, ELISA (VEGF-A protein) and immunocytochemistry (VEGF receptors). Proliferation of UM cells incubated with neutralizing anti-VEGF-A antibody bevacizumab (≤ 2.5 mg/ml), or VEGF-A (≤ 100 ng/ml) was assessed by BrdU ELISA. It was further measured by real-time PCR, whether VEGF-A (100 ng/ml) modulated the expression ratio of VEGF-A itself and its antiangiogenic antagonist pigment epithelium-derived factor (PEDF). All UM cells expressed VEGF-A, VEGF-R1, VEGF-R2 mRNA and protein. In each cell line, the proliferation was stimulated by VEGF-A or inhibited by blocking VEGF-A, or both: Bevacizumab significantly decreased the proliferation in MEL-270 ($p=0.005$), OMM-2.3 ($p=0.001$), and OMM-2.5 ($p=0.011$). Increased VEGF-A signaling significantly raised the proliferation in MEL-270, OM-431 ($p<0.001$, respectively), and OMM-2.3 ($p=0.043$) in a dose-dependent manner, but did not significantly change the VEGF-A/PEDF mRNA expression ratio. Autocrine VEGF-A signaling seems to be present in UM sustaining the proliferation of both primary and metastatic UM

cells. Apparently, VEGF-A signaling in UM cells does neither react on VEGF-A expression in the sense of a feedback loop, nor contribute to an proangiogenic shift of the VEGF-A/PEDF ratio.

IRB Status: None

Disclosures:

KONRAD KOCH, MD: No financial relationships to disclose

O202

IMMUNOLOGICAL TREATMENTS FOR UVEAL MELANOMA

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COMPREHENSIVE CANCER CENTER, WAKE FOREST UNIVERSITY

That immunotherapy can be effective in treating uveal melanoma is supported by a number of studies in animal tumor models. Several clinical observations also suggest that the immune responses regulate uveal melanoma progression. To date, however, uveal melanoma has been refractory to immunotherapeutics. Several approaches are now being implemented to improve immunotherapy for uveal melanoma. Existing immunotherapeutic regimens are being more rationally applied using tumor genetic criteria to better identify patients. The treatment of advanced cutaneous melanoma has been revolutionized with the development of the cytotoxic T-lymphocyte antigen-4 inhibitor, ipilimumab, and this and other immune "check-point" inhibitors, such as those targeting the Program Death-1 pathway, are under investigation. Vaccines have been an attractive though not yet clinically approved therapy for melanoma. Dendritic cell vaccines have shown promise and are being tested in clinical trials. Immune modulation with epigenetic drugs is also under investigation, as are combining immunotherapeutics with conventional chemotherapy and radiotherapy and with targeted approaches. Recent studies have led to an improved understanding of the regulation of immune responses in uveal melanoma. Cellular immune suppressors, including regulatory T cells and myeloid derived suppressor cells, and several soluble immune suppressors have been identified that are potential therapeutic targets. There are challenges. There are differences in the immunology of uveal and cutaneous melanoma, and extrapolations between the two are tenuous. New biomarkers that can help monitor treatment response are necessary. Uveal melanoma is a rare cancer, and novel clinical trial designs are also necessary. Participation in well designed, scientifically sound, clinical trials is essential.

Disclosures:

PIERRE TRIOZZI, MD: No financial relationships to disclose

O203

TOWARDS T CELL-BASED IMMUNOTHERAPY OF UVEAL MELANOMA

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DEPARTMENT OF INTERNAL MEDICINE 5, HEMATOLOGY AND ONCOLOGY, UNIVERSITY OF ERLANGEN-NUREMBERG

Uveal melanoma metastases predominantly arise in the liver and universally remain fatal. Novel therapies are being explored for their effectiveness against uveal melanoma metastases and immunotherapy may be a potential option for high-risk patients in the adjuvant setting. Uveal melanoma may be particularly responsive to T cell-based immunotherapy, because it originates in the immune-privileged eye. The localization of the primary tumor in the immune-privileged eye excuses the tumor cells from continuous, peripheral immunological pressure. This may render primary uveal melanoma more immunogenic, than tumor cells from non-privileged sites and allow expression of novel tumor antigens to which the patient's endogenous T cell repertoire is not tolerized. The clinical and genetic differences between cutaneous, including conjunctival, melanoma and uveal melanoma underscore the need for immunotherapy specifically designed for uveal melanoma patients. Furthermore, the analysis of tumor lymphocyte infiltration may provide novel insights into T cell responsiveness to these distinct subsets of melanomas. In this presentation, the current developments in the field of immunotherapy for uveal melanoma are reviewed and novel data on in-vitro T cell activation to uveal melanoma will be presented.

IRB Status: Approved

Disclosures:

JACOBUS BOSCH, MD, PHD: No financial relationships to disclose

IM04 – Ocular Infection and Host Defense

O204

C-TYPE LECTIN RECEPTORS: DYSFUNCTION OF HOST DEFENSE IN THE EYE?

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VETERANS ADMINISTRATION MEDICAL CENTER AND OREGON HEALTH AND SCIENCE UNIVERSITY, MOLECULAR MICROBIOLOGY AND IMMUNOLOGY¹; LABORATORY OF IMMUNOLOGY, NEI, NIH²

Although inflammation is essential for host defense against infection, its dysregulation contributes to a variety of inflammatory and autoimmune disorders. We are interested in how host defense mechanisms triggered by innate immune receptors may participate in autoimmunity targeted to the eye, modeled in experimental autoimmune uveoretinitis (EAU). The ability of the Toll-like receptors (TLRs) to direct adaptive immune responses and to potentiate uveitogenic T cell responses is known, yet deficiency in TLRs 2/4/3/9 does not significantly reduce EAU, suggesting that these TLRs may be dispensable for induction of EAU. Thus, the innate immune pathways involved in triggering EAU remain poorly understood. Using multiplex array analysis of isolated neuroretina we identified early induction of genes involved in fungal and mycobacterial host defense responses, including cytokines and macrophage chemotactic factors, along with *Dectin-1*, *Dectin-2*, and *Mincle*, which comprise a subgroup of C-type lectin receptors (CLRs) that transduce signals through Syk- and CARD9-dependent

pathway. *Card9* expression was essential for induction of this transcriptional response and contributed functionally to uveitis because EAU was significantly reduced in *CARD9* KO mice as evaluated by fundus and histopathologic grading. The protective effects of *CARD9*-deficiency coincided with reduced T cell proliferation and an impaired Th17 effector response. In attempts to identify the CLR upstream responsible for triggering *CARD9*-signaling response, EAU was induced in the single-gene KO mice: Dectin-1, Dectin-2, and Mincle. Fundoscopic and histopathologic evaluations support the role for Mincle over that of Dectin-1 or Dectin-2 for induction of EAU and suggest that deficiency in Mincle partially explains the *CARD9*-dependency. Just as CLRs have emerged as dominant immune signaling responses in infections, these findings indicate that the pathogenesis of uveitis includes innate signaling pathways involving CLRs and the *CARD9* signaling pathway as a central mechanism in ocular autoimmune responses.

IRB Status: None

Disclosures:

HOLLY ROSENZWEIG, PHD: No financial relationships to disclose

O205

GENERATION OF A HIGHLY EFFICACIOUS VACCINE AGAINST OCULAR HSV-1 INFECTION

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UNIVERSITY OF OKLAHOMA HEALTH SCIENCES CENTER¹;
SOUTHERN ILLINOIS UNIVERSITY SCHOOL OF MEDICINE, MEDICAL MICROBIOLOGY²

Conservatively, over 1 billion individuals worldwide are latently infected with herpes simplex virus type 1 (HSV-1) with 20% of those infected experience some form of reactivation of the virus. Within the eye, reactivation can have serious consequences including blindness for which the virus is the leading cause of infectious corneal blindness in the industrialized world. Currently, there is no successful vaccine to the pathogen. We have generated a mutation in the ICP0 gene (deletion of the ICP0 nuclear localization signal or Δ NLS) to render the HSV-1 Δ NLS highly susceptible to interferon. The efficacy of the HSV-1 Δ NLS vaccine strain was compared to a Herpes-vac-like vaccine (amino acids 1-302 of HSV-2 gD-2 adjuvanted with alum and monophosphoryl lipid A). Using C57BL/6J (WT) mice vaccinated with the HSV-1 Δ NLS or HSV-2 gD-2 and challenged with an LD₅₀ of HSV-1 strain McKrae, we found both vaccines prevented neovascularization and protected challenged mice from virus-mediated mortality but the HSV-1 Δ NLS vaccine reduced viral loads in the cornea and trigeminal ganglion two- and four-logs greater than mice vaccinated with the HSV-2 gD-2 vaccine. By comparison, only the HSV-1 Δ NLS vaccine protected mice deficient in a functional type I interferon response to challenge with HSV-1 in terms of virus titer and cumulative survival. Even though neovascularization was elevated in comparison to that found in the cornea of vaccinated WT mice, the levels were significantly below that found in non-vaccinated WT mice. Currently, we are exploring the contribution of each arm of the adaptive immune response (i.e., B and T cells) in the efficacy of the HSV-1 Δ NLS vaccine.

In conclusion, the HSV-1 Δ NLS holds great promise in providing significant efficacy against ocular HSV-1 infection in terms of tissue pathology and establishment of latency in response to exposure to the highly neurovirulent McKrae strain of HSV-1.

IRB Status: None

Disclosures:

DAN CARR, PHD: No financial relationships to disclose

O206

INTRACELLULAR *P. AERUGINOSA* VERSUS CORNEAL EPITHELIAL CELL: WHO WINS AND WHY?

SUZANNE FLEISZIG¹, David Evans²

UC BERKELEY¹; TOURO UNIVERSITY CA COLLEGE OF PHARMACY, BIOLOGICAL SCIENCES²

Corneal epithelial barrier function against microbes is generally thought to involve surface mucins, junctional barriers, and extracellular antimicrobials. Additionally, we have found that corneal epithelial cells can behave as "non-professional phagocytes," i.e. they can internalize bacteria and then suppress their viability within acidified lysosomes. The importance of this potential innate defense against microbes has not been appreciated for epithelial cells at any body site. Our data show that MyD88 participates in phagocytic activity of corneal epithelial cells. Unfortunately, we have found that the potentially devastating corneal pathogen *P. aeruginosa* can thrive in these cells, by evading acidified lysosomes and establishing replicative niches inside plasma membrane blebs. While lysosome evasion and "bleb-niche" formation both require the bacterial type III secretion system (T3SS), these capacities are separable. Lysosome evasion depends on the ADP-ribosylation (ADPr) activity of the T3SS effector ExoS, but not PopB, one of two T3SS translocators. In stark contrast, both ExoS and PopB are needed for *P. aeruginosa* to establish bleb-niches. Phagocytic activity of corneal epithelial cells and bacterial evasion of this defense are both novel concepts. Understanding the host-pathogen interactions that result in these very different outcomes, could lead to new strategies for tipping the outcome in favor of the host.

IRB Status: None

Disclosures:

SUZANNE FLEISZIG, OD, PHD: Consultant/Advisor relationship with Allergan; Contracted Research relationship with Alcon; Contracted Research relationship with Allergan

O207

MECHANISMS OF *TOXOPLASMA GONDII* INFECTION OF HUMAN RETINA?

IRA BLADER

UNIVERSITY AT BUFFALO

Toxoplasma gondii is the causative agent of ocular toxoplasmosis, which is a common and potentially blinding infectious disease of the posterior retina. The prevalence of this infection

is directly due to the parasite's ability to establish a chronic infection and reside within a quiescent cyst that is impervious to the host's immune response and currently prescribed anti-parasitic drugs. Occasionally a cyst in the retina or other tissue reactivates and the released parasites will replicate until the host mounts a properly regulated immune response. If parasite growth cannot be controlled or if the immune response is not properly regulated, then disease will develop. How anti-*Toxoplasma* immune responses are generated and how parasites replicate within the retina are largely unanswered questions. Our lab has been investigating both of these issues. First, we have identified that the endosomal Toll-Like Receptor, TLR11, is critically required to detect parasites in the murine retina. But unlike other tissues, we find that retinal TLR11 differentially signals to activate MHC Class II expression and to trigger the recruitment of neutrophils and inflammatory monocytes. Next, we are studying how *Toxoplasma* replicates within the low oxygen environment of the retina. Our data has indicated that this is accomplished by the parasite activating the host oxygen sensing transcription factor Hypoxia Inducible Factor-1 and that this transcription factor is critically required for parasite growth. We have further demonstrated that *Toxoplasma* needs host HIF-1 signaling to promote host glycolytic gene expression and that the parasite induces host glycolytic metabolism in a HIF-1-dependent manner.

IRB Status: Verified

Disclosures:

IRA BLADER, PHD: No financial relationships to disclose

IM05 – Pathogenic Antibodies in Ocular Disease

O208

INTRODUCTION TO PATHOGENIC ANTIBODIES IN OCULAR DISEASE

JOHN CURNOW

UNIVERSITY OF BIRMINGHAM

The role of autoantibodies in a number of autoimmune diseases is well established. For those that affect the eye this includes autoimmune retinopathy and thyroid eye disease, although the exact specificity of the antibodies and the pathogenic mechanism operating to cause the damage remains an area of intense investigation. In addition, more recent evidence suggests that antibodies may play pathogenic roles in other ocular diseases including glaucoma, ocular surface disease and age-related macular degeneration. The speakers in this session will provide examples of how pathogenic antibodies may contribute to ocular disease.

IRB Status: None

Disclosures:

JOHN CURNOW, PHD: Consultant/Advisor relationship with Celentyx Ltd.

O209

AUTOREACTIVE T AND B CELLS IN DRY EYE DISEASE

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ALLERGAN INC., BIOLOGICAL SCIENCES¹; IOBA - UNIVERSITY OF VALLADOLID, OPHTHALMOLOGY²; UT SOUTHWESTERN MEDICAL SCHOOL, OPHTHALMOLOGY³; BAYLOR COLLEGE OF MEDICINE, OPHTHALMOLOGY⁴

Dry eye is a prevalent ocular surface disease defined by localized inflammation and tissue destruction that results in ocular discomfort, fatigue and chronic pain, accompanied by blurred and fluctuating vision. Recent data strongly support the theory that dry eye is a self-antigen-driven autoimmune disease. During desiccating stress (DS)-induced experimental dry eye disease, acute cytokine production, activation of resident ocular surface derived antigen presenting cells, and subsequent activation of autoreactive lymphocytes is required for the initiation and development of disease. Indeed, dry eye disease can be adoptively transferred to nude recipient mice by CD4+ T cells isolated from the regional lymph nodes of mice with experimental dry eye. However, antigen presenting cell (APC)-depleted mice exposed to DS do not develop full-blown dry eye disease, and CD4+ T cells isolated from these mice are not pathogenic in nude recipients. In addition to autoreactive T cells, autoantibodies derived from self-reactive B cells also appear to contribute to ocular surface disease. Autoantibodies against Kallikrein (Klk) 13 were identified in serum from dry eye mice, but were undetectable in naïve controls. Autoantibody-containing serum or purified IgG from dry eye mice was sufficient to mediate complement-dependent ocular surface inflammation. For example, passive transfer of purified IgG from dry eye mice resulted in decreased tear production (60.9±10.0% of baseline) compared to recipients of control IgG (143.2±29.4%), and yielded a significant ($p<0.05$) increase in Gr1+ neutrophils (52.4±11.2 vs. 21.9±5.7), which correlated with reduced goblet cell numbers (49.6±7.6) compared to mice receiving control IgG (78.4±4.3). Complement C3b deposition was detected in recipients of dry eye-specific IgG, and complement-depleted mice were resistant to IgG-mediated disease. Collectively, these data indicate that autoreactive lymphocytes, including T and B cells, contribute to the immunopathogenesis of experimental dry eye, and suggest that autoantibody deposition contributes to the predominantly T cell-mediated ocular surface disease.

IRB Status: None

Disclosures:

MICHAEL STERN, PHD: Employee relationship with Allergan, Inc.

O210

AUTOANTIBODIES TO INSULIN-LIKE GROWTH FACTOR-1 RECEPTOR IN THYROID-ASSOCIATED OPHTHALMOPATHY

JOHN CURNOW, Matthew Edmunds

UNIVERSITY OF BIRMINGHAM

Thyroid-Associated Ophthalmopathy (TAO) is an inflammatory condition of the eye socket that occurs in 30-50% of those with autoimmune thyroid disease, particularly Graves' disease (GD). Autoantibodies to insulin-like growth factor-1 receptor (IGF-1R) have been implicated in TAO pathogenesis. Previous studies determined that GD and TAO serum (but not healthy control; HC) triggers orbital fibroblast T cell chemo-attractant and inflammatory mediator production, and that these effects were abrogated by an IGF-1R monoclonal antibody. Additionally, GD/TAO serum displaced radiolabelled IGF-1 from orbital fibroblasts. We developed two novel ELISA to detect and quantify serum IGF-1R autoantibodies in GD and TAO. The two assays reliably and reproducibly detected monoclonal IGF-1R antibody, and were not affected by the presence of recombinant IGF-1 or non-IGF-1R monoclonal antibody. However, despite extensive analysis we have been unable to demonstrate any differences in IGF-1R antibody levels between any groups. Interestingly, similar results were recently reported by two other groups. The effects of anti-IGF1R autoantibodies were also thought to impact on IGF-1R expression on T cell subsets. Although we confirmed that IGF-1R was differentially expressed on CD4+ and CD8+ T cell memory populations, being highest on naïve T cells and sequentially decreasing with differentiation to central and effector memory status, we could find no difference between GD, TAO and HC groups when analysing each state of T cell differentiation. We did however find highly significant changes in peripheral blood T cell populations, with reduced cytokine-producing effector memory T cells and increased naïve T cells, in both CD4+ and particularly CD8+ T cells. Collectively these data cast doubt on a simple model of anti-IGF-1R autoantibodies being responsible for ocular pathology associated with Graves' disease.

IRB Status: Approved

Disclosures:

JOHN CURNOW, PHD: Consultant/Advisor relationship with Celentyx Ltd.

O211

AUTOANTIBODIES IN GLAUCOMA

FRANZ GRUS

EXP OPHTHALMOLOGY, UNIVERSITY MEDICAL CENTER

In glaucoma, the elevated intraocular pressure cannot explain the disease in all patients. However, the pathogenesis of the disease is widely unknown. Biomarker research could be help to understand the disease process. Beside some genetic and proteomic biomarkers, immunoproteomics could play a significant role. Several studies could provide hints for an involvement of autoantibodies in the pathogenesis of the disease. In several studies we could demonstrate consistent up- and down-regulations of immune reactivities

against ocular antigens in glaucoma patients. These findings could lead to a better understanding of the pathomechanisms involved in glaucoma, but could also lead to new innovative ways of early detection and neuroprotection of the disease.

IRB Status: Approved

Disclosures:

FRANZ GRUS, MD PHD: No financial relationships to disclose

O212

ROLE OF ANTI-RETINAL AUTOANTIBODIES IN PARANEOPLASTIC RETINOPATHY

GRAZYNA ADAMUS

OREGON HEALTH AND SCIENCE UNIVERSITY

Visual paraneoplastic syndromes, including cancer-associated retinopathy (CAR) and melanoma-associated retinopathy (MAR) represent uncommon retinal disorders associated with different systemic malignant tumors and anti-retinal AAbs. Our current findings show the associations of lung cancer in 33% of total seropositive patients, breast malignancy in 20% and melanoma in 14% of patients. Autoimmunity is an important causative factor in the initiation and progression of retinal degeneration. There is experimental and clinical evidence for the role of AAbs in retinal pathology. The molecular mechanism of CAR/MAR includes a 2-step AAb involvement: 1) *through an anti-tumor response*, involving an immune response that is elicited against aberrantly expressed antigens in tumor; 2) *through an anti-retinal response*, involving the AAb penetration through retinal layers and their internalization by target cells. We showed that AAbs block the target-antigen metabolic function and causes an increase in intracellular calcium leading to the induction of apoptosis. Thus, the increase in intracellular calcium may play a key role in the antibody-induced retinal apoptosis and retinal degeneration. Also, degenerating retina provided antigenic material that perpetuated the immune responses, which in effect can contribute to pathogenic process. Anti-retinal AAbs of different specificities were cytotoxic to retinal cells in vitro and in vivo. Passive transfer of anti-recoverin AAbs triggered apoptosis of photoreceptor cells and caused an influx of macrophages into the retina. Normal Abs were not toxic to retinal cells. Retinal degeneration in CAR is a continuing process until complete photoreceptor death. We also showed that multiple AAbs seem to be more relevant for etiology and diagnosis of CAR. Knowledge of the full autoantibody repertoire in retinopathy is an important requirement in better understanding of the autoimmune process to facilitate better diagnosis, prognosis, and treatment.

IRB Status: Approved

Disclosures:

GRAZYNA ADAMUS, PHD: No financial relationships to disclose

PH01 – Vertebrate and Invertebrate Visual Systems: Lessons from Evolution

O213

THE EVOLUTIONARILY CONSERVED SNARE PROTEIN, GOS28, IN *DROSOPHILA*

NANSI COLLEY, Erica Rosenbaum, Eva Vasiljevic,
Spencer Cleland, Carlos Flores

UNIVERSITY OF WISCONSIN-MADISON

The *Drosophila* Golgi SNARE protein, Gos28, displays 43% identity with human Gos28. Here, we demonstrate that Gos28 plays a critical role in the vesicular transport of rhodopsin during its biosynthesis. Null mutations in *gos28* cause a defect in the targeting and transport of rhodopsin to the rhabdomeres. In the *gos28* mutant, we detect an accumulation of an immature, hyper-glycosylated form of rhodopsin in the secretory pathway. Furthermore, this immature rhodopsin is insensitive to treatment with endoglycosidase H (Endo H), providing evidence that rhodopsin has successfully reached the cis-Golgi. However, because rhodopsin fails to undergo trimming events that occur later in the Golgi, our data suggest that rhodopsin does not effectively exit the trans-Golgi. These results indicate that Gos28 is required for transport events between the sub-compartments of the Golgi. In order to study the importance of the conserved SNARE domain in Gos28, *in vivo*, we used site-directed mutagenesis and generated a collection of transgenic flies expressing Gos28 mutations. We studied animals expressing Gos28 that lack its transmembrane domain or animals that harbor one of several different point mutations in the conserved SNARE domain. Our mutant analyses have provided information on residues that are important for Gos28's function as a SNARE protein during vesicle fusion and rhodopsin biosynthesis. Additionally, we demonstrate that mutations in *gos28* lead to a late-onset retinal degeneration. Our work highlights the importance of SNARE proteins in rhodopsin maturation as well as for photoreceptor cell survival and provides insights into mechanisms of retinal degeneration.

IRB Status: None

Disclosures:

NANSI COLLEY, PHD: No financial relationships to disclose

O214

EVOLUTION OF TRP CHANNELS: A LESSON FROM MUTATION AND CONSERVATION OF *DROSOPHILA* TRPL PORE DOMAIN

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MEDICAL NEUROLOGY

Transient receptor potential (TRP) channels constitute a large superfamily of polymodal channel proteins with diverse roles in many transduction and sensory pathways. The TRP superfamily, which is conserved through evolution, consists of seven subfamilies and its members are expressed in many cell types. These channels participate in most sensory modalities and they either open directly in response to ligands or physical stimuli, or indirectly, downstream of a signal transduction cascade. We explored the role of critical amino acids in the highly conserved pore region of the *Drosophila* TRP and TRPL by generating specific point mutations, which affect the activation state of the channel and may explain how the channel-lipid interactions determine the activation state of the channel. The alignment of transmembrane region five (S5) of TRP channels reveals highly conserved amino acids. Amino acids in this conserved region may be important for preventing constitutive activity of the channel that is observed in heterologous expression of TRPC channels and may be critical for the effects of lipids on the channel. To get insights into the gating mechanism and validate homology model, we bioinformatically analyzed TRPL. We searched for comutating amino acids, as well as amino acids shown to be of importance in other TRP channels. We identified the area surrounding the semi-conserved amino acid F557 in S5 as a critical regulator of TRPL activity. By a series of mutations we generated TRPL mutants which became spontaneously active in HEK293 cells, or showed different responses to the non-specific TRP modulator linoleic acid (a polyunsaturated fatty acid). By mutating amino acid pairs we were able to gain insights in the orientation of the transmembrane domains and validate homology model. We conclude that despite sequence differences and evolutionary separation, the gating mechanism and overall structure of TRPC and TRPV channels is conserved.

IRB Status: None

Disclosures:

BARUCH MINKE, PHD: No financial relationships to disclose

O215

A JOURNEY TO THE EVOLUTIONARY ORIGINS OF ANIMAL VISION

TODD OAKLEY

UC SANTA BARBARA

The evolutionary origins of complex innovations like eyes are contentious because they involve multiple, interacting components, which may have differing evolutionary histories. Research in our laboratory uses phylogenetic techniques to investigate separately the individual components of complex systems like animal phototransduction, to elucidate their origins. Phototransduction underlies animal vision, utilizing numerous proteins to translate light information into nervous impulses. The keystone protein of phototransduction is opsin, and we want to understand when and how it originated, leading to the question – what are the

most distant relatives of humans to possess opsins? We have studied opsin and other phototransduction components in cnidarians like *Hydra magnipapillata*. Similarities with humans and other bilaterian animals indicates a shared common ancestry of phototransduction at least since humans and cnidarians diverged. Recent genome sequences from fungi uncovered opsin-like sequences, which could push opsin origins to before animals. However, our phylogenetic analyses and behavioral experiments suggest that most of those fungal genes may not be opsins. Our recent work uses constraints from the fossil record to provide estimates of the absolute timing of major events in opsin history. We find that phototransduction arose in the common ancestor of cnidarian plus bilaterian animals and this origin involved mutation of an existing GPCR protein to become the first opsin. Other components of phototransduction pre-date this opsin origin, and were likely involved in a different sensory function, indicating that phototransduction arose in part by co-option of existing components. The close relationship of opsins with melatonin receptors suggests the possibility of a common, perhaps ancestral, response to light because melatonin is broken down by light and could serve as an indirect signal of light levels.

IRB Status: None

Disclosures:

TODD OAKLEY, PHD: No financial relationships to disclose

O216

EVOLUTION OF VERTEBRATE PHOTOTRANSDUCTION AND THE RETINOID CYCLE: CLUES FROM AGNATHANS

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The molecular mechanisms underlying phototransduction, as well as those underlying the retinoid cycle, are well understood in vertebrates. For both systems, the molecular components are remarkably conserved across jawed vertebrates. In order to study the evolution of these systems, it is important to understand the comparable molecular components and mechanisms in agnathans; i.e. the jawless vertebrates comprising extant lampreys and hagfish, that diverged from jawed vertebrates around 500 million years ago. Although there is some limited information about such components and mechanisms in a few lamprey species, very little is known about the processes or components in hagfish or in other lampreys. Current knowledge of agnathan photoreceptors, from anatomical, electrophysiological, and comparative studies, will be reviewed. New data from the eye transcriptome of the hagfish, *Eptatretus cirrhatus*, and the southern hemisphere lamprey, *Geotria australis*, generated by high-throughput sequencing, sheds light on the molecular mechanisms of phototransduction and retinoid cycling in agnathan species, and provides clues to the evolution of phototransduction, photoreceptors, and the retinoid cycle in vertebrates.

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IRB Status: International

Disclosures:

TREVOR D. LAMB, SCD: No financial relationships to disclose

PH02 – Retinoids and the Vitamin A Cycle

O217

IRBP AND THE VERTEBRATE RETINA

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OPHTHALMOLOGY DEPARTMENT, EMORY UNIVERSITY

Interphotoreceptor retinoid-binding protein (IRBP) is a soluble protein synthesized in photoreceptors. It occupies the subretinal space and binds retinoids and fatty acids. It may provide a reducing environment and protect ligands. In evolution, IRBP appeared at the same time as the vertebrate eye. The IRBP gene arose from an internal quadruplication of a smaller ancestral single-repeat gene that may not have functioned in vision. Seeking the evolutionary origin of IRBP, we tested ancestral IRBP genes from early- and pre-vertebrates. Lamprey has the classic 4-repeat IRBP gene structure but amphioxus does not. The latter contains one repeat, and has at least 8 exons. We ask if the lancelet ortholog has the same visual role as mammalian IRBP, a function that remains unresolved. Developmentally, IRBP controls axial growth. When IRBP is missing (in the KO mouse), the eye grows too long at P8 and results in a myopic shift of 15 diopters in the KO vs. WT at P90. Globe enlargement coincided with remodeling of INL at P7-P12. Inner rods were reduced at P7 in the KO vs. WT. The effects of missing IRBP are selective with direct causal link to myopia. However, later degeneration of PhRs commencing abruptly at P23 appears unrelated to earlier myopic eye growth. Dopamine (DA) from inner retina slows axial growth (PNAS 1989; 86:704-6). We predicted IRBP and DA interact to regulate eye growth. We measured retinal levels of DA, its metabolite DOPAC, and number of tyrosine hydroxylase (TH)+ cells in IRBP KO and WT retinas. DA and DOPAC were increased in KO vs. WT. TH+ cells were more numerous in the KO vs. WT. Previous studies show a decrease in DA in experimental vision-dependent myopia, but our results demonstrated that eye elongation before eye-opening may be controlled by different mechanisms from later experimental myopia.

IRB Status: None

Disclosures:

JOHN NICKERSON, PHD: No financial relationships to disclose

O218

STRUCTURAL AND FUNCTIONAL BASIS OF RPE65 CATALYTIC MECHANISM

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LRCMB, NATIONAL EYE INSTITUTE, NIH

The retinal pigment epithelium (RPE) retinoid visual cycle isomerase RPE65 is a unique member of the carotenoid oxygenase family that does not oxidatively cleave carbon-carbon double bonds but instead has evolved into an all-trans retinyl ester (RE): 11-cis retinol isomerase. Implicit in this description are both an isomerase activity and an O-alkyl ester cleavage function, suggesting a complex mechanism. To date, the isomerase functionality, supplying 11-cis retinol is the better characterized component, with the ester cleavage component less well so. This is despite the fact that the primary bond cleavage activity of RPE65 is the O-alkyl cleavage. The non-heme iron cofactor is thought to play a role in the O-alkyl cleavage. Previously, we showed that the aromatic side chain-rich environment of the RPE65 substrate-binding cleft is responsible for favoring an 11-cis specific outcome, and mutation of many of these residues decreases 11-cis ROL synthesis but increases 13-cis isomer due to reduced stringency. These data also support a carbocation/radical cation mechanism of retinol isomerization. To explore other aspects of the mechanism, we have investigated the role of RPE65 palmitoylation, an area of contention in RPE65 biochemistry; C112 is identified as being palmitoylated in RPE65. Different groups confirm or deny the existence of C112 palmitoylation. By labeling experiments we detected a very low level of RPE65 palmitoylation, negating a putative structural role such as in rhodopsin, but favoring a dynamic metabolic role. Given the apparent ability of RPE65 to be both a cytosolic and a membrane associated protein, a dynamic palmitoylation may help explain this situation, in addition to the disparate findings on the existence of RPE65 palmitoylation.

IRB Status: None

Disclosures:

T. MICHAEL REDMOND, PHD: No financial relationships to disclose

O219

A2E AND LIPOFUSCIN

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MEDICAL UNIVERSITY OF SOUTH CAROLINA¹; VANDERBILT UNIVERSITY²

The accumulation of lipofuscin in the retinal pigment epithelium (RPE) is a hallmark of aging in the eye. Lipofuscin is primarily characterized by fluorescence. The best characterized component of lipofuscin is A2E, a fluorescent *bis*-retinoid by-product resulting from the normal visual process. The absorption of a photon by the visual pigments generates all-trans retinal, which can in turn be processed into A2E. In organic solvent extracts of lipofuscin from mice and humans, A2E is the most abundant molecule that is observed by classical chromatographic techniques. Indeed, *in vitro*, A2E has been shown to exhibit a broad spectrum of cytotoxic effects. Our studies have correlated the distribution of lipofuscin and A2E across the human and mouse RPE. Lipofuscin fluorescence was imaged in the RPE from human donors of various ages and from assorted mouse models. The spatial distribution of A2E was determined using matrix-assisted laser desorption-ionization imaging mass

spectrometry on both flat-mounted and transversally sectioned RPE tissue. Our data support the clinical observations in humans of strong RPE fluorescence, increasing with age, in the central area of the RPE. However, there was no correlation between the distribution of A2E and lipofuscin, as the levels of A2E were highest in the far periphery and decreased toward the central region. Interestingly, in all the mouse models, A2E distribution and lipofuscin fluorescence correlate well. These data demonstrate that the accumulation of A2E is not responsible for the increase in lipofuscin fluorescence observed in the central RPE with aging in humans. Other potential candidates including possible A2E adducts are being investigated.

IRB Status: Verified

Disclosures:

ROSALIE K. CROUCH, PHD: No financial relationships to disclose

O220

ATOMIC STRUCTURES OF THE RETINOID CYCLE PROCESSING ENZYMES

KRZYSZTOF PALCZEWSKI

CASE WESTERN RESERVE UNIVERSITY

Photon-induced isomerization of 11-cis-retinal to all-trans-retinal is at the heart of our ability to perceive light. This event initiates a series of conformational changes in opsin pigments followed by a cascade of protein-protein interactions and enzymatic activities within photoreceptor cells of the retina together called phototransduction. To maintain vertebrate vision, the spent all-trans-retinal chromophore released from rhodopsin in photoreceptor cell outer segments must be converted back to 11-cis-retinal, a process largely accomplished in an adjacent layer of the retina called the retinal pigmented epithelium (RPE). The key enzymes involved in this process are ABCA4 transporter, retinoid isomerase (RPE65), esterifying enzyme call LRAT and retinol dehydrogenases. In addition, a number of retinol-binding proteins are involved in this process. Structural studies significantly increased our understanding of the retinoid cycle. Pharmacology combined with structural biology of these enzymes holds great promise for developing innovative therapies targeting retinal diseases.

IRB Status: Approved

Disclosures:

KRZYSZTOF PALCZEWSKI, PHD: No financial relationships to disclose

O221

CAN RODS REGENERATE THEIR PIGMENT WITHOUT THE RPE?

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WASHINGTON UNIVERSITY SCHOOL OF MEDICINE

Rapid regeneration of visual pigment following its destruction by light is critical for the function of mammalian cones as

daytime photoreceptors. This is made possible by the retina visual cycle, which uses Müller cells to rapidly convert the all-*trans* retinol released from photoreceptors into 11-*cis* retinol. A key feature of the retina visual cycle is that it regenerates pigment only in cones, which can oxidize 11-*cis* retinol to 11-*cis* retinal by an unknown dehydrogenase, but not in rods. The mechanisms restricting this pathway to cones are not well understood. The *Nr2e3* knockout (*rd7*) mouse presents a unique opportunity to investigate this issue as its retina has hybrid rods with some cone-like morphological features in addition to expressing a subset of cone genes. We investigated, whether the *rd7* retina can promote rod pigment regeneration independently of the pigment epithelium by performing single-cell and transretinal recordings as well as microspectrophotometric measurements from *rd7* rods. Our results demonstrate that, paradoxically, the hybrid rods in *rd7* retina can undergo pigment regeneration and dark adaptation in the absence of pigment epithelium. We conclude that the altered morphology and/or gene expression of the hybrid *rd7* rods enable them to access the cone-specific retina visual cycle and use it for pigment regeneration. The access to the rapid retina visual cycle accelerates dramatically the dark adaptation of *rd7* rods in vivo. At the same time, the competition between cones and *rd7* rods for chromophore recycled by the retina visual cycle results in a delay of cone dark adaptation in the *rd7* retina. Morphological and molecular comparison of wild type and *rd7* rods is underway in order to determine the mechanisms that enable wild type cones and *rd7* rods, but not wild type rods, to utilize the retina visual cycle for pigment regeneration.

IRB Status: Approved

Disclosures:

VLADIMIR KEFALOV, PHD: No financial relationships to disclose

PH03 – Photoreceptor Physiology and Adaptation

O222

SENSITIVITY RECOVERY OF CONE PHOTORECEPTORS UNDER CONTINUOUS ILLUMINATION: DIFFERENCES IN FISH AND MONKEY RETINAS FIT BY A SINGLE SIMULATION MODEL

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UNIVERSITY OF CALIFORNIA SAN FRANCISCO, OPHTHALMOLOGY

The absolute light sensitivity of retinal cone photoreceptors depends on the intensity of background illumination. Starting at background onset, light adaptation in cones consists of two components with distinct time courses. A rapid component is completed within about one second and is characterized by a loss of light sensitivity to flashes superimposed on the background in both mammalian and non-mammalian species. A second component develops over many seconds and is characterized by a paradoxical recovery of light sensitivity under the continuing background; its features are different in mammalian and non-mammalian species. In zebrafish and goldfish, sensitivity recovery manifests as both a slow drift

towards its starting value of the response to the background itself and as an increase in the absolute sensitivity to flashes superimposed on the background. The sensitivity recovery has an exponential time course of 3 sec time constant in zebrafish and 14 sec in goldfish. In the macaque monkey, however, sensitivity recovery is manifested only as a slow drift in the response to the background light while the absolute sensitivity to superimposed flashes is unchanged. Sensitivity recovery involves Ca^{2+} -dependent modulation of the ligand sensitivity of cGMP-gated ion channels since we have shown that recovery does not occur in the absence of CNG-modulin/EML1, the protein that mediates the ion channel modulation and is expressed in mammalian and non-mammalian cones alike. A mathematical model of the cone phototransduction pathway that includes Ca^{2+} -dependent modulation of CNG ion channels and numerous other known mechanisms of Ca^{2+} modulation robustly simulates both the rapid and slow components of light adaptation, including the difference in sensitivity recovery between fish and monkey, but only when a slow, transient intracellular flux of free Ca^{2+} ions from the inner to the outer segment cytoplasm is assumed to occur at the onset of background light.

IRB Status: None

Disclosures:

JUAN KORENBROT, PHD: No financial relationships to disclose

O223

MOLECULAR MECHANISMS THAT CONTROL RESPONSE KINETICS IN MOUSE RODS, SOME UNUSUAL SUSPECTS

MICHAEL WOODRUFF¹, Ching-Kang Chen², Daniel Tranchina³, Gordon Fain¹

UCLA, INTEGRATIVE BIOLOGY AND PHYSIOLOGY¹; VIRGINIA COMMONWEALTH UNIVERSITY, BIOCHEMISTRY AND MOLECULAR BIOLOGY²; NEW YORK UNIVERSITY, COURANT INSTITUTE OF MATHEMATICS AND DEPARTMENT OF BIOLOGY³

We previously showed (Chen et al. J. Neurosci. 32:15998-16006, 2012) that over-expression of rhodopsin kinase (RKox) or recoverin deletion (*Rv*^{-/-}) accelerated dim-flash response recovery (τ_{REC}) and bright-flash response recovery (τ_{D} ; dominant time constant), indicating that RK and Rv, which control Rh* turnoff, may also control cyclic GMP phosphodiesterase (PDE*) turnoff. We have reduced GTPase activating protein (GAP) expression to slow PDE* deactivation, to separate it further in time from the relatively rapid Rh* deactivation, and we have re-tested RKox and *Rv*^{-/-} effects. If RK and Rv influence only Rh* turnoff, the RKox and *Rv*^{-/-} acceleration of recovery should be diminished/eliminated in the slowed PDE* decay rods, a critical test that they also target PDE. We looked at two animals: 1) RGS9-1 anchoring protein heterozygous, *R9AP*^{+/-}, which expresses the anchoring protein and the other GAP complex proteins, RGS9-1 and Gbeta5L, at 50% of wild-type (WT) levels; and 2) *R9AP*^{+/-}; *RGS9-1*^{+/-} (*GAPux*), which expresses GAP complex proteins at 30% of WT. Both *R9AP*^{+/-} and *GAPux* showed an increase in τ_{REC} and τ_{D} , indicating that decreased GAP expression indeed slowed PDE* decay. When *R9AP*^{+/-} or *GAPux* were combined with RKox or *Rv*^{-/-} τ_{REC}

and τ_D decreased to near WT values. Time constants (msec) were (τ_{REC} , τ_D): WT, 197 ± 10 , 173 ± 10 ; R9AP $^{+/-}$, 255 ± 18 , 254 ± 18 ; R9AP $^{+/-}$ RKox, 212 ± 21 , 182 ± 10 ; R9AP $^{+/-}$ Rv $^{-/-}$, 176 ± 13 , 187 ± 17 ; GAPux, 316 ± 42 , 320 ± 23 ; GAPuxRKox, 187 ± 21 , 202 ± 17 ; and GAPuxRv $^{-/-}$, 207 ± 19 , 216 ± 18 . These differences were significant (Student's t-test, $p < 0.05$). The results show that RKox or Rv $^{-/-}$ can have large effects on response decay. The most likely explanation is that RK and Rv can modulate PDE* decay, in addition to the role of they play in Rh* phosphorylation/inactivation. These inactivation steps, essential for shaping the rod light response, likely interact. Modulation of PDE* may have an important role in adjusting response kinetics during light adaptation.

IRB Status: None

Disclosures:

MICHAEL WOODRUFF, PHD: No financial relationships to disclose

O224

MODULATION OF PHOTOTRANSDUCTION AND PHOTORECEPTOR CELL SURVIVAL IN KV2.1 POTASSIUM CHANNEL KNOCKOUT MICE

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UNIVERSITY OF CALIFORNIA, DAVIS, DEPARTMENTS OF OPHTHALMOLOGY, VISION SCIENCE AND CELL BIOLOGY, HUMAN ANATOMY¹; UNIVERSITY OF CALIFORNIA, DAVIS, DEPARTMENTS OF NEUROBIOLOGY, PHYSIOLOGY, AND BEHAVIOR²

Apart from conducting electrical signals, neuronal voltage gated potassium (K⁺) channels also help to regulate cell volume, intracellular pH, and apoptosis. Kv2.1, which belongs to the shab subfamily of the voltage gated K⁺ channels, is highly expressed in photoreceptors. In oocytes, recordings show that homotetramer of Kv2.1 fail to conduct outward current; however, the physiological function of Kv2.1 in photoreceptors remains undefined. One hypothesis is that the outward conductance through Kv2.1 completes the circulating dark current loop and generates the light-induced hyperpolarization. To test this, our study investigates the consequences of deleting Kv2.1 on light signaling and photoreceptor survival. Light evoked changes in membrane currents were evaluated in rods of both wild type (WT) and in mice lacking Kv2.1 (KO) using suction electrodes. Flashes of light suppressed the circulating current in both WT and KO rods, indicating that another outward conductance could partially substitute that of the Kv2.1. In addition, KO flash responses showed a prominent, rapid undershoot, the duration of which increased as the flash strength increased, suggesting that this compensatory current might be regulated by cyclic nucleotides. Corneal electroretinography recordings revealed a dramatic decline in the a-wave amplitude with age, yet sections from KO retinas showed no clear sign of photoreceptor degeneration in early adulthood. Immunohistochemistry of retinal sections confirmed high immunoreactivity for the Kv2.1 subunits in the inner segments of photoreceptors of WT mice. No fluorescence was detected in the outer segments or the outer nuclear layer. All apparent immunoreactivity for

Kv2.1 was lost in the KO. These results show that the inward cGMP current of the outer segment is balanced by the outward cation conductance generated by Kv2.1 K⁺ channels and another unknown source(s). This alternate conductance is not wholly sufficient and appears to be under developmental or age-related regulation.

IRB Status: None

Disclosures:

SYED ABBAS, PHD: No financial relationships to disclose

O225

CHARACTERIZATION OF DARK NOISE IN ROD PHOTORECEPTORS AND IMPLICATIONS FOR VISUAL THRESHOLD

ALAPAKKAM SAMPATH, Johan Pahlberg

UNIVERSITY OF CALIFORNIA, LOS ANGELES

Near absolute threshold, sensory systems face the problem of maximizing their sensitivity under conditions where the stimulus is sparse or weak, and signal amplification is essential. However, amplification is only beneficial if signals can be amplified more efficiently than the system's intrinsic noise. Two forms of dark noise in the rod photocurrent may interfere with the ability to signal single photon absorptions; infrequent discrete noise resembling single-photon responses, and continuous lower amplitude noise that is present in every rod. We generated transgenic mice where these forms of rod noise are reduced, respectively; rods with reduced rhodopsin (Rh^{+/−}) and reduced cGMP phosphodiesterase (rd1^{+/−}) expression. Rhodopsin concentration in Rh^{+/−} bred into a C57Bl/6J background was reduced by ~50%. Expressed PDE6 in rd1^{+/−} mice was reduced by ~50%. We further bred Rh^{+/−} mice into a GCAPs^{−/−} background, allowing the easier resolution of single-photon responses due to the elimination of Ca²⁺ dependent feedback in phototransduction. Membrane current noise was measured using either suction electrodes on rod outer segments, or patch electrodes ($V_m = -40$ mV) on rod cell bodies in retinal slices. We found that discrete noise events in Rh^{+/−} rods were reduced by ~2-fold compared to Rh^{+/+} demonstrating their origin in thermal activation of rhodopsin. The discrete noise rate was 0.013 s^{-1} (n=68) in Rh^{+/+} and 0.006 s^{-1} (n=39) in Rh^{+/−} rods. Power spectra calculated from dark noise in rd1^{+/−} mice displayed ~50% reduction in power for the bandwidth up to 10 Hz. No reduction in power was observed in mice heterozygous for rod transducin, demonstrating the origin of continuous noise in spontaneous PDE activation. Despite the reduction in dark noise in these mouse models, no differences were seen in the amplitude or time course of light-evoked responses. These models are suitable to establish the role of these forms of noise in setting visual threshold.

IRB Status: Approved

Disclosures:

ALAPAKKAM SAMPATH, PHD: No financial relationships to disclose

O226**ADAPTIVE POTENTIATION IN ROD PHOTORECEPTORS****TIMOTHY KRAFT, Alex McKeown***UNIVERSITY OF ALABAMA AT BIRMINGHAM, VISION SCIENCES*

Photoreceptors adapt to changes in illumination by altering transduction kinetics and sensitivity. We describe a new form of rod photoreceptor adaptation that manifests as a response potentiation, a paradoxical hypersensitivity following periods of light exposure. Following the conditioning periods of saturating illumination, mouse rods exhibit a 10-35% increase in circulating dark current, varying with the duration of conditioning saturation. Increased sensitivity grows with exposure times up to three minutes, and decreases for longer exposures. The response potentiation is very short lived; cells return to their initial dark-adapted sensitivity with a time constant of 6.8 s. Rods from a mouse expressing cyclic nucleotide gated channels incapable of binding calmodulin showed a 60% adaptive potentiation (AP) upon returning to darkness. Halving the extracellular magnesium concentration prolonged the adaptation, increasing the time constant of recovery to 13.3 s, but did not affect the magnitude of potentiation. In GCAP-/- mice AP was large (68±8%) recovered quickly ($\tau = 4.3$ s), and the recovery was unaffected by reduced Mg concentration. Application of an insulin-like growth factor-1 receptor (IGF-1R) kinase inhibitor (Tyrphostin AG1024) blocked the adaptive potentiation, while application of an insulin receptor (IR) kinase inhibitor (HNMPA(AM)₃) failed to block potentiation. The broad-acting tyrosine phosphatase inhibitor orthovanadate also blocked the potentiation. Adaptive Potentiation (AP) was also found in rat and monkey rod photoreceptors. These findings establish a unique form of adaptation in rod photoreceptors showing transient hypersensitivity, and are consistent with a model in which light history, acting via the IGF-1 receptor can increase the sensitivity of rod photoreceptors, while the photocurrent overshoot is regulated by Ca-calmodulin and Mg-sensitive guanylate cyclase activating proteins (GCAPs).

IRB Status: Verified

Disclosures:

TIMOTHY W. KRAFT, PHD: No financial relationships to disclose

PH04 – Defects in Ion Channel Function Causing Disease**O227****RESTORATION OF VISUAL FUNCTION TO BLIND MICE WITH A CHEMICAL PHOTOSWITCH TARGETED TO DEGENERATED RETINA****IVAN TOCHITSKY¹, Aleksandra Polosukhina¹, Vadim Degtyar¹, Nicholas Gallerani¹, Caleb Smith¹, Aaron Friedman¹, Russell Van Gelder², Dirk Trauner³, Daniela Kaufer¹, Richard Kramer¹***DEPARTMENT OF MOLECULAR AND CELL BIOLOGY, UC BERKELEY¹; UNIVERSITY OF WASHINGTON²; LUDWIG MAXIMILIAN UNIVERSITY OF MUNICH³*

Degenerative blinding diseases such as retinitis pigmentosa and age-related macular degeneration affect millions of patients around the world. These disorders cause the progressive loss of rod and cone photoreceptors from the retina, while sparing the remaining amacrine, bipolar and retinal ganglion cells (RGCs). Our goal is to develop a pharmacological means of restoring light sensitivity to the blind retina, by converting some of these remaining types of retinal neurons into artificial photoreceptors. To achieve this goal, we have created several small molecule chemical "photoswitches" that can be used to control the activity of retinal neurons by reversibly blocking native ion channels in response to light. In order to evaluate the ability of these photoswitches to restore light sensitivity to blind animals, we have tested them in mouse models of retinitis pigmentosa.

Here, we present the restoration of light sensitivity to blind mice in vitro and in vivo with a photoswitch molecule called DENAQ. DENAQ photosensitizes the degenerated retina to white light about as bright as ordinary daylight. The molecule persists in the eye for several days after intravitreal injection with no apparent toxicity. We identify RGCs as the primary cell type photosensitized by DENAQ. DENAQ does not affect the photoreceptor-mediated light response of wild-type mouse retinas and does not photosensitize wild-type RGCs. We have identified the class of ion channels upregulated in the degenerated retina - HCN channels - which are responsible for DENAQ's disease-selective photosensitization of RGCs. Intravitreal injection of DENAQ also restores light sensitivity to blind mice in vivo in an exploratory locomotory behavior and enables visual learning in a visual cued fear conditioning assay.

We believe that DENAQ and similar molecules are promising potential drug candidates for treating degenerative blinding diseases and will continue to test their safety and efficacy in other animal models of retinal degeneration.

IRB Status: Approved

Disclosures:

IVAN TOCHITSKY, PHD: No financial relationships to disclose

O228**DISRUPTION IN KIR7.1 CHANNEL LEADS TO BLINDNESS****BIKASH PATNAIK, Pawan Shahi, Xinying Liu, Nathan York, Simran Brar, Wenxiang Liu, De-Ann Pillers***UNIVERSITY OF WISCONSIN*

Within the retina, retinal pigment epithelium (RPE) and photoreceptors share a small volume of subretinal space. Subretinal space integrity is the primary responsibility of the RPE cells. When the retina is illuminated an immediate key change in the subretinal space is the decrease in $[K^+]_o$ from 5 mM dark level to 2 mM light exposed. Inwardly rectifying potassium channel (Kir7.1) line the apical processes of RPE cell and respond to restore subretinal space $[K^+]$ to its dark level. In general, ion-channels serve many functions so genetic mutations of ion-channel cause disease. This talk will focus on recent discoveries of blindness caused by defective Kir7.1 channel in the retina. Cytoplasmic mutation p.R162W causes autoso-

mal dominant snowflake vitreoretinal degeneration (SVD). Kir7.1 channel current is potentiated by external Rb^+ and are inhibited by high concentration of Cs^+ or Ba^{2+} . R162W mutation resulted in a dysfunctional channel which was not responsive to Rb^+ nor Cs^+ . We found that SVD mutation result in a dominant negative loss of Kir7.1 function. The mutant protein was accumulating in the ER compared to localization of wild-type protein in the plasma membrane when ectopically expressed. Several non-sense and mis-sense mutations lead to autosomal recessive Lebers congenital amaurosis (LCA). We present our finding that the non-sense mutation results in a truncated protein which does not locate to the membrane and hence no Kir current. Further inhibition of mice Kir channels *in vivo* resulted in a suppression of ERG waveforms similar to what has been reported in Kir7.1 channelopathy. We provide molecular insight of blindness due to Kir7.1 channelopathy.

IRB Status: None

Disclosures:

BIKASH PATNAIK, PHD: No financial relationships to disclose

O229

ROLE OF MECHANOSENSITIVE TRP CHANNELS IN RETINAL PATHOLOGY

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UNIVERSITY OF UTAH SCHOOL OF MEDICINE, DEPARTMENT OF OPHTHALMOLOGY AND VISUAL SCIENCES, MORAN EYE CENTER

The aim of this research was to identify the molecular mechanisms by which pathological forces are transduced into neuronal and glial pathology within the retina. Elevated intraocular pressure (IOP) is a major risk factor for glaucoma, a blinding disease involving retinal ganglion cell (RGC) degeneration and reactive Müller gliosis. Glaucomatous progression also involves RGC swelling, followed by pre-apoptotic shrinkage in the peripheral retina, where RGC loss predominates. Approved glaucoma treatments target IOP lowering; however, uncovering the mechanisms by which IOP activates glia and injures RGCs might lead to complementary neuroprotective treatments. To examine mechanosensors, dissociated retinal cells from Thy1:CFP mice were exposed to different tonicities (140-490 mOsm). Volume and calcium were measured with calcein, fura-2 and electrophysiology. IOP was elevated by injected microbeads and measured tonometrically. Radial stretch (0.5-2 Hz and 0.5-10%) was applied to plated cells. Viability was analyzed with IHC, TUNEL, Annexin V, and Live/Dead assays. RGCs and Müller glia are intrinsically responsive to osmotic pressure and substrate stretch. These mechanosensitive responses are largely mediated by the cation channel TRPV4. Calcium influx through TRPV4 exacerbates cell swelling, potentially via cytoskeletal reorganization. Selective TRPV4 stimulation is sufficient to induce RGC loss *in vitro* and *in vivo*. TRPV4 is required for RGC death from both prolonged hypotonic stimulation in retinal wholemounts and radial stretching in dissociated preparations. TRPV4 inhibition also prevents neurodegeneration in a mouse model of glaucoma. Furthermore, TRPV4 activation is sufficient to trigger the MAPK path-

way and reactive gliosis in Müller glia. This effect was potentiated by ablation of TRPC1/3 channels. In summary, mechanical activation of TRPV4 may underlie calcium dysregulation, glial reactivity, cell swelling and neurodegeneration associated with mechanical stress within the mammalian retina. The sensitivity of retinal glia and/or neurons to biomechanical forces might be additionally modulated through heteromerization with other TRP isoforms.

IRB Status: None

Disclosures:

DANIEL RYSKAMP, BS: Equity Owner relationship with Asha Vision, LLC; Patents/Royalties relationship with Asha Vision, LLC

O230

VOLTAGE DEPENDENT CALCIUM CHANNELS IN STEM CELL DERIVED RETINAL PIGMENT EPITHELIUM

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TAMPERE UNIVERSITY OF TECHNOLOGY AND BIOMEDITECH
DEPARTMENT OF ELECTRONICS AND COMMUNICATIONS ENGINE

Retinal pigment epithelium (RPE) has an impressive calcium machinery controlling its many different functions including transepithelial transport of ions and water, dark adaption of photoreceptor activity, phagocytosis, secretion, and differentiation. We investigated this machinery in human embryonic stem cell (hESC) derived RPE cells which provide a potential therapeutic approach for the treatment of several degenerative eye diseases. In this study, we characterized the expression and functionality of the voltage dependent calcium channels in hESC-RPE by whole cell patch clamp recordings and high resolution microscopy. Our RPE cells were differentiated from the pluripotent embryonic cell lines which were derived and maintained using existing protocols in the Skottman laboratory. The differentiation of RPE was performed in the Skottman laboratory as described before. The calcium currents were characterized by whole-cell patch clamp recordings of mature hESC-RPE monolayers showing strong pigmentation and typical cobblestone morphology. The expression and localization of different voltage gated calcium channels was investigated by confocal microscopy. Our recordings demonstrated a heterogeneous pattern of voltage-dependent calcium channels in hESC-RPE. Some cells showed sustained high-voltage-activated currents resembling L-type calcium channels whereas other cells showed transient currents typical to T-type calcium channels. With confocal microscopy we were able to demonstrate the presence of L-type channels and at least two different T-type channels. Our results are consistent with the studies of fresh and cultured RPE from multiple species in the literature. However, to our knowledge they have not been confirmed on stem cell derived RPE before.

IRB Status: None

Disclosures:

SOILE NYMARK, PHD: No financial relationships to disclose

0231**RESPONSE OF THE VISUAL PATHWAYS IN THE HUMAN BRAIN TO GENE THERAPY**

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Subjects with Leber's congenital amaurosis due to RPE65 mutations who had previously received subretinal injection of an adeno-associated virus (AAV) carrying the normal hRPE65 cDNA (AAV2-hRPE65v2) and who met the inclusion criteria were invited to participate in functional and structural imaging of the brain post-intervention. Functional imaging was done with functional magnetic resonance imaging (fMRI). Structural imaging employed diffusion tensor imaging (DTI) in order to examine the effect of deprivation and subsequent unilateral retinal gene therapy on the organization and/or reorganization of white matter microstructure in V1. Tractography was also used to examine the effect of deprivation and unilateral gene therapy on the integrity and/or plasticity of white matter fiber bundles connecting V1 to all other primary and higher order visual centers in the brain. Finally, correlations between the results of the functional and structural data evaluated the relationship between structural and functional changes brought about by gene therapy. The results reveal that visual experience initiated by gene therapy not only enhances functional responses, but also may be responsible for the reorganization and maturation of synaptic connectivity of the visual pathway. Despite severe and long-term visual impairment, LCA-RPE65 patients have intact visual pathways which become responsive after treatment and the structural changes correlate with the region of retina that is exposed to the gene therapy reagent.

This study was supported by R21EY020662 from the National Eye Institute by the Center for Cellular and Molecular Therapeutics (CCMT) at CHOP, the Foundation Fighting Blindness-sponsored CHOP-PENN Pediatric Center for Retinal Degenerations, Clinical Translational Science Award NIH/NCRR UL1-RR-024134, 1R01EY019014-01A2, Research to Prevent Blindness, the Paul and Evanina Mackall Foundation Trust at Scheie Eye Institute, anonymous donors, the Italian Telethon Foundation, and the F. M. Kirby Foundation.

IRB Status: Not provided

Disclosures:

JEAN BENNETT, MD, PHD: No financial relationships to disclose

0232**GENE THERAPY FOR RETINOSCHISIS/PROGRESS IN HUMAN GENE THERAPY**

PAUL SIEVING

NATIONAL EYE INSTITUTE, NIH

X-linked retinoschisis (XLRs) is a developmental retinal genetic disease that impairs vision in men from childhood and progresses with age. The condition was first recognized clinically in 1898 and is readily diagnosed by the characteristic intraretinal macular cysts arranged in a "spoked-wheel" pattern surrounding the foveal. Additionally, the electroretinogram exhibits a characteristic reduction of the b-wave relative to a preserved a-wave, which implicates functional pathology involving the proximal retina. However, the cellular understanding of the condition lagged until the gene, retinoschisin, was identified about 15 years ago. Genetic analysis led to probing the molecular basis of the disease, based on the structure and function of the retinoschisin protein. Several murine models of XLRs have been created, and surprisingly, the mouse has revealed new insights of the clinical extent and course of human XLRs disease. Mutations in the human retinoschisin gene have been correlated with severity of the human XLRs phenotype. Introduction of normal human retinoschisin cDNA into the *Rs1* knockout mouse improves retinal structure and neural function and provides proof-of-concept that gene replacement therapy is a plausible treatment for XLRs. Work is underway to develop human gene therapy clinical trials.

IRB Status: Approved

Disclosures:

PAUL SIEVING, MD, PHD: No financial relationships to disclose

0233**ONE YEAR FOLLOW-UP OF A PHASE 1 GENE THERAPY TRIAL WITH SUBRETINAL RAAV.SFLT-1 FOR THE LONG-TERM TREATMENT OF WET AGE-RELATED MACULAR DEGENERATION**

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One-year post-administration, we report the safety and preliminary efficacy of a single subretinal injection of rAAV.sFlt-1.

Prior to enrolment, majority of subjects required extensive treatment with ranibizumab (average 18 anti-VEGF injections) for wet age-related macular degeneration (wAMD). Six subjects received 100 µl injection of rAAV.sFlt-1 (3 low dose: 10E10 vg and 3 high dose: 10E11 vg; 2 control subjects were treated only with protocol ranibizumab). All subjects received 0.5 mg ranibizumab at baseline and Day 30. During the follow-up period, subjects were allowed retreatment with ranibizumab according to strict, masked criteria based on visual acuity (VA) and optical coherence tomography (OCT). Laboratory tests included hematology, renal and hepatic function, electrolytes, urine protein and IgM, IgG, IgA and lymphocyte subset analysis. In addition, assays for anti-AAV antibodies, neutralizing antibodies, and ELISpot were also performed. Ophthalmic safety was assessed by biomicroscopic examination, IOP, indirect ophthalmic examination, OCT, color fundus photography and fluorescein angiography. Neutralizing antibody and clinical laboratory assessments, including blood biochemistry, complete blood count and lymphocyte subsets, generally remained unchanged from baseline. There was no evidence of loss of visual acuity, IOP elevation, retinal detachment, or intraocular or systemic inflammation. SD OCT demonstrated a decrease or lack of fluid in all subjects. Out of a possible 72 rescue injections for the subjects in the treatment group, two were given; 4/6 (67%) subjects required no retreatments, and 2 subjects required 1 rescue injection. Control subjects received 10X as many rescue treatments during the criteria-driven PRN period. The average VA in treatment group was 41.8 and 49.3 EDTRS at baseline and at one year, respectively. None of the subjects showed signs of choroidal or retinal atrophy. These results confirm that rAAV.sFlt-1 subretinal injection is safe and well tolerated and a single injection of rAAV.sFlt-1 may provide a durable, long-term treatment option for wAMD.

IRB Status: International

Disclosures:

ELIZABETH RAKOCZY, PHD: Consultant/Advisor relationship with Avalanche Biotechnologies

O234

EXPLORATION OF AAV-MEDIATED GENE THERAPIES FOR INHERITED OCULAR DISORDERS

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TRINITY COLLEGE DUBLIN, SCHOOL OF GENETICS AND MICROBIOLOGY

Recombinant adeno associated virus (AAV) is being used in the development of gene therapies for many inherited ocular diseases. The success of AAV as a vector for gene therapy is related to its good safety profile and its ability to target a broad range of cell types employing naturally occurring and engineered serotypes. Recent clinical trials for Leber congenital amaurosis (LCA) and Choroideremia using AAV for gene delivery have provided evidence that this vector is well tolerated in the human eye subsequent to subretinal administration. Recent findings from our team exploring AAV-delivered gene therapies for two inherited ocular disorders, that is, mitochondrially

inherited Leber Hereditary Optic Neuropathy (LHON) and autosomal dominantly inherited rhodopsin-linked Retinitis Pigmentosa (RHO-adRP) will be outlined. Results associated with administration via subretinal delivery of a dual component AAV-based therapy for RHO-adRP encompassing RNA interference (RNAi)-mediated suppression of rhodopsin in conjunction with rhodopsin gene replacement in animal models will be presented. Issues such as use of differing AAV serotypes and co-administration of two vectors will be addressed. In addition results relating to therapeutic strategies being explored for LHON will be outlined. Data associated with intravitreal administration of an AAV2/2 vector incorporating a complex I substitute as a means to compensate for the complex I deficiency characteristic of LHON will be discussed. With regard to LHON, studies relating to a cell based approach for this disorder will be referred to. On-going studies employing next generation sequencing (NGS) to characterize the Irish inherited retinal degeneration patient population will be briefly overviewed.

IRB Status: None

Disclosures:

GWYNETH JANE FARRAR, PHD: Director relationship with Genable

O235

GENE THERAPY FOR USHER 1B: STUDY ON MYO7A ISOFORMS AND DOMINANT MUTATIONS

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Usher 1B is a genetic disorder characterized by congenital deafness and progressive blindness, which results from lack of functional MYO7A. MYO7A is an unconventional myosin, with a tail made up of two tandem repeats units (MyTH4-FERM) responsible for cargo binding and auto-regulation of the molecule. Human MYO7A encodes two long transcripts: IF1 and IF2, which differ by a 38-amino acid sequence that is present in the first FERM domain of IF1 but not IF2. LV and AAV-based gene therapy approaches have reported success in treating MYO7A-null mice, but, so far, no attention has been paid to the isoform introduced, or treating mutations that may not be loss-of-function. Here, we addressed these issues by determining the transcription levels of both IF1 and IF2 in the retina and their functionality in vitro and in vivo. We also studied the retina of the Hdb mouse, which manifests dominant inheritance of cochlear and vestibular dysfunction. Our results indicate that most of the transcripts of MYO7A correspond to IF2, in both the RPE and photoreceptors. IF2 also seems to be functionally more competent than IF1 in rescuing melanosome motility characteristic in MYO7A-null RPE primary cultures, despite both resulting in vivo phenotype correction. Studies of heterozygous HDB retinas indicated the presence of a dominant-negative mutant MYO7A. Hence, not all MYO7A mutations may cause loss-of-function, demonstrating that simple gene replacement cannot be regarded as a universal Usher 1B treatment. Where gene replacement is applicable, our studies show that DNA, encoding MYO7A IF2, should be used.

IRB Status: Verified

Disclosures:

VANDA LOPES, PHD: No financial relationships to disclose

PH06 – Novel Engineered Therapies for Glaucoma

0236

GLAUCOMA THERAPY BY CONTACT LENSES WITH VITAMIN E BARRIERS

ANUJ CHAUHAN

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Conventional ophthalmic drug by eye drops is very inefficient with a low bioavailability of 1-5%. Our research has shown that the corneal bioavailability can be increased to about 50% by using drug eluting contact lenses. Currently available commercial contact lenses are however not optimal for drug delivery due to the very short release duration of about an hour for most ophthalmic drugs. Our lab has focused on developing contact lenses that can deliver ophthalmic drugs for significantly longer durations compared to commercial lenses. This talk will describe our recent approach of using Vitamin E diffusion barriers for attenuating drug release from contact lenses. The Vitamin E barriers are created by soaking a silicone hydrogel contact lens in a solution of Vitamin E in ethanol, and then extracting the ethanol in water. The loaded Vitamin E precipitates into high aspect ratio barriers with a thickness of about 5 nm and diameter of about 200 nm. The barriers are very effective in increasing the release duration of most drugs, with a more pronounced effect for hydrophilic drugs. For some drugs, we have succeeded in increasing the drug release duration to as long as a few months. The drugs of interest include timolol, dorzolamide, latanoprost, cysteamine, dexamethasone, dexamethasone phosphate, cyclosporine, lidocaine, fluconazole, etc. We have also successfully conducted animal studies in a glaucoma model of Beagle dogs to prove that the contact lenses can safely reduce the intraocular pressure at significantly lower drug loadings compared to eye drops. We also demonstrated that extended delivery of drug from Vitamin E loaded contact lenses can achieve therapeutic reduction in the IOP for durations significantly longer than the wear duration through creation of drug depots in the eyes. This opens the possibility of achieving continuous therapeutic effects with intermittent use of the lenses.

IRB Status: Verified

Disclosures:

ANUJ CHAUHAN, PHD: No financial relationships to disclose

0237

EYEWATCH: A NOVEL ADJUSTABLE GLAUCOMA DRAINAGE DEVICE

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LHTC, EPFL¹; JULES GONIN EYES HOSPITAL, LAUSANNE²; GLAUCOMA CENTER MONTCHOISI CLINIC³

Purpose: This work reports on the *in vivo* testing of a novel non-invasively adjustable glaucoma drainage device (AGDD), which features an adjustable outflow resistance, thereby allowing the regulation of the intraocular pressure (IOP) on a per patient basis.

Methods: Under general anesthesia, the AGDD was implanted on 10 white New-Zealand rabbits under a scleral flap and set in an operationally closed position in a way analogous to the Ex-PRESS® device. The IOP was measured on a regular basis on both the operated and the control eyes using a rebound tonometer. Once a month the AGDD was adjusted non-invasively from its fully closed position to its fully open position and the resulting pressure drop was measured. In group 1 the contralateral eye was not operated and served as control. In group 2, an Ex-PRESS® P-50 was implanted in the contralateral eye to serve as comparative reference. After sacrifice, the eyes were collected for histology evaluation.

Results: The mean preoperative IOP was 11.1 ± 2.4 mmHg. In group 1 the post-operative IOP was significantly lower for the operated eye (6.8 ± 2 mmHg) compared to the non-operated eye (13.1 ± 1.6 mmHg) during eight days after surgery. In group 2 a difference in IOP in the early postoperative period between the AGDD (closed position) and the Ex-PRESS® was present but did not reached a statistical significance ($p = 0.1$). When opening the AGDD using the control unit, the IOP dropped significantly from 11.2 ± 2.9 mmHg down to 4.8 ± 0.9 mmHg ($p < 0.05$) for both groups.

Conclusion: Critical aspects such as safety and efficiency of the AGDD were assessed in this first *in vivo* study. The fluidic resistance was non-invasively adjustable during the post-operative period with the AGDD at its fully closed and open positions.

IRB Status: Approved

Disclosures:

SYLVAIN ROY, MD, PHD: No financial relationships to disclose

0238

CAN REPLACEMENT OF DAMAGED OR LOST TRABECULAR MESHWORK CELLS RESTORE IOP CONTROL IN GLAUCOMA?

MARKUS KUEHN

THE UNIVERSITY OF IOWA

Failure to maintain the intraocular pressure (IOP) at healthy levels is a significant risk factor for the development of glaucoma. Controlling elevated IOP remains an effective way to minimize vision loss. While the mechanisms that lead to the development of elevated IOP in primary open angle glaucoma (POAG) have not been fully elucidated, it is clear that dysfunction and death of trabecular meshwork cells contribute to the development of the disease.

It is conceivable that replacing damaged or lost trabecular meshwork cells could restore function to the tissue and reestablish IOP control. In order for such an approach to be clinically feasi-

ble, cells to be used for transplantation must be easily obtained, plentiful, and preferably derived from the patient herself in order to avoid immune responses. We believe that trabecular meshwork cells derived from pluripotent stem cells (iPSC) provide an ideal solution. Our findings demonstrate that iPSC—arisen from both mouse and adult human fibroblasts—can be induced to closely resemble primary trabecular meshwork cells in many important aspects. These cells, termed iPSC-TM, resemble primary TM cells morphologically, express many TM cell markers, and develop the ability to phagocytose material.

Thus far transplantation of mouse iPSC-TM into the anterior chamber of Tg-MYOC^{437H} mice has been highly encouraging. iPSC-TM take up residence within the trabecular meshwork and were detectable at the end of the experiment, four weeks after injection. The formation of intraocular tumors is a potential concern, but thus far no eyes with teratomas have been observed. Importantly, transplantation did result in a significant decrease in IOP in a majority of treated animals.

While still at an early stage, these data are very encouraging and suggest that it may be possible to restore lost functionality to the trabecular meshwork through cellular treatments.

IRB Status: Verified

Disclosures:

MARKUS KUEHN, PHD: No financial relationships to disclose

O239

TRABECULAR MESHWORK STEM CELLS AND CELL-BASED THERAPY FOR GLAUCOMA

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Reduced cellularity within the trabecular meshwork (TM) is observed in glaucoma patients and correlates with elevated intraocular pressure (IOP). Using stem cells to replenish the remaining TM cells may facilitate restoration of the TM function and control IOP. We previously described that multipotent stem cells from TM (TMSCs) have the ability to differentiate into phagocytic TM cells. These cells can home to the TM region after been transplanted into mouse anterior chamber. In current study, we investigate the specific niche of TMSCs and the potential of TMSCs for cell-based therapy for glaucoma to reconstruct the outflow pathway.

Brumodeoxyuridine (BrdU) was injected into newborn mice peritoneally. Up to 12 weeks of chasing period, BrdU retaining cells were detected in the insert region of the TM and co-expressed stem cell marker OCT4. Laser photocoagulation was used to decrease the TM cellularity and damage the outflow structure on C57BL/6 mice. Mouse IOP was elevated up to 24 weeks. Elevated IOP resulted in optic nerve damage evidenced by decreased photopic negative response (PhNR) by electroretinography and axonal loss by toluidine blue staining. Most of the eyes had no synechia in the anterior chamber angle confirmed by optical coherence tomography and histology. Decreased TM cellularity and disorganized extracellular matrix was detected by transmission electron microscopy. After

injection of TMSCs, laser damaged TM region was repaired and IOP decreased. When treated with dexamethasone or TGFβ2, TMSCs survived and remained proliferation.

This study shows that TMSCs are resistant to glaucoma-associated factors and have the ability to repair damaged TM tissue. This indicates the potential of cell-based therapy for glaucoma.

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IRB Status: None

Disclosures:

YIQIN DU, MD, PHD: No financial relationships to disclose

O240

INTRAVITREAL NEURAL STEM CELLS FROM HUMAN ADULT TEMPORAL LOBES ARE NEUROPROTECTIVE IN A RAT MODEL OF OPTIC NERVE TRANSACTION

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Progressive degeneration of retinal ganglion cells (RGCs) is a major pathologic feature of optic neuropathies including glaucoma. We investigated the neuroprotective effect of intravitreal human neural stem cells (hNSCs) on the survival of RGCs in the rat retina with optic nerve transection (ONT). On the day of intraorbital ONT, intravitreal injections of 100,000 hNSCs, which were obtained from primary cultures of adult human temporal lobe tissues, 100,000 apoptotic hNSCs after freeze and thaw, and the same volume of vehicle were done. We found that intravitreal hNSCs significantly increased the survival of RGCs at day 7 after ONT compared to control. The human specific real time RT-PCR assay for mRNA for human GAPDH to follow the fate of the intravitreally administered hNSCs indicated that more than 20% of the intravitreally administered hNSCs were recovered after 1 and 5 days. However, no cells were found to be integrated into the rat retina by histologic analysis. To test the hypothesis that the increased survival of RGCs were explained by the secretory neuroprotective factors from the injected hNSCs, expression of brain derived neurotrophic factor (BDNF) and stanniocalcin-1 (STC-1) were assayed by real time RT-PCR. RT-PCR revealed that STC-1 was significantly up-regulated in eyes at day 1 after ONT, but BDNF was not. The results suggested that intravitreal injection of hNSCs or secretory factors from hNSCs might be a useful therapy for optic nerve diseases in which RGCs undergo apoptosis.

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IRB Status: Approved

Disclosures:

SANG JIN KIM, MD: No financial relationships to disclose

Ocular Imaging

OI01 – Image-Based Outcome Measures for Treatment of Retinal Disease

O241

FUNDUS AUTOFLUORESCENCE IMAGING

MICHEL MICHAELIDES

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INSTITUTE OF OPHTHALMOLOGY

The utility of fundus autofluorescence imaging as an outcome metric for treatment trials will be critically discussed with reference to Stargardt Disease, Retinitis Pigmentosa and Age-Related Macular Degeneration.

IRB Status: International

Disclosures:

MICHEL MICHAELIDES, BSC, MB, BS, MD(RES), FRCOPHTH,
FACS: No financial relationships to disclose

O242

OPTICAL COHERENCE TOMOGRAPHY PATTERN OF CENTRAL PHOTORECEPTOR IMPAIRMENT IN PATIENTS WITH RETINITIS PIGMENTOSA

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Mouafk Asaad¹**

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CATALUNYA²

Retinitis pigmentosa is a slowly progressive inherited retinal disease. The patients experience reduced visual function caused by degeneration of photoreceptors and retinal pigment epithelium. At the end stage of the disease, the loss of central cones leads to blindness.

High definition optical coherence tomography (HD-OCT Cirrus Carl Zeiss Meditec, Dublin, CA, USA) was used to characterize photoreceptor impairment in central retina of 42 eyes of 21 patients with nonsyndromic retinitis pigmentosa (NSRP) without retinal oedema or hemorrhage. External limiting membrane (ELM), photoreceptor inner and outer segment junctions (IS/OS), and cone outer segment tips (COST) were identified and classified in 4 categories (0=absent; 1=abnormally structured; 2= short highly reflective line; 3=reflective line with normal length). Presence of foveal bulge (FB) was also assessed.

Abnormal patterns were always disclosed in the three studied layers. ELM showed grade 2 in 28 (66.67%), and grade 1 in 11 (26.19%) been absent in 3 eyes (7.14%). IS/OS displayed grade 2 in 26 (61.90%), grade 1 in 12 (28.57%) and grade 0 in 4 eyes (9.53%). COST was absent in 33 (78.57%) revealing grade 2

only in 3 eyes (7.14%), and grade 1 in 6 eyes (14.29%). FB was inexistent in 38 eyes (90.48%).

COST has been described as the first retinal layer that becomes disorganized but sometimes is difficult to identify. FB indicates a domelike appearance of the IS/OS junction in healthy central retina due to the higher number of membranous discs of central cones. This clearly identifiable feature was the most frequently altered in NSRP and could indicate early damage of central photoreceptors in an easy and reliable manner.

IRB Status: None

Disclosures:

BARBARA DELAS, MD: No financial relationships to disclose

O243

ELLIPSOID ZONE WIDTH ANALYSIS OF OCT

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Purpose: Here we report rate of progression in EZ width in patients with RPGR-mediated x-linked Retinitis Pigmentosa (xLRP). The edge of the EZ is a transition zone between relatively healthy and relatively degenerate retina (Hood et al, Biomed Optics Exp, 2011). We therefore also determined whether decline in visual field sensitivity is greater when measured in the transition zone than when measured at other locations in the visual field.

Methods: Measures were obtained from 44 patients (ages 8 to 30 yrs) with xLRP due to a mutation in the RPGR gene. On five consecutive yearly visits, static perimetric fields were obtained with a Humphrey field analyzer. Beginning with the third visit, horizontal midline SD-OCT scans were obtained with a Heidelberg Spectralis HRA +OCT. Based on the segmented scans, two nasal and two temporal field locations were selected for each patient. One nasal and temporal pair was just inside the EZ edge and the other was just outside the EZ edge. The rate of change at these locations was compared to the rates for the macula (5 points within central 10 degrees) and the mid-periphery (70 points between 10 and 30 degrees).

Results: The average yearly decrease in EZ width in patients with RPGR-mediated xLRP was 0.86° (248 mm). Visual sensitivity declined at the highest rate just inside (0.8 ± 0.1 dB/year; 18%) and just outside (0.9 ± 0.1 dB/year; 19%) the edge of the EZ. By comparison, sensitivity in the macula and mid-periphery declined at significantly slower at rates of 0.4 ± 0.1 dB/year (9%; t=23.5, p<0.001) and 0.6 ± 0.07 dB/year (13%; t=14.1, p<0.001).

Conclusions: EZ width declines rapidly in patients with RPGR-mediated xLRP. The identification of the EZ facilitates targeted visual field testing at transition zone sites with the highest rate of progression.

IRB Status: Approved

Disclosures:

DAVID BIRCH, PHD: No financial relationships to disclose

O244

REAL-TIME MONTAGING OF ADAPTIVE OPTICS RETINAL IMAGES

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Adaptive optics (AO) systems allow in vivo examination of cone photoreceptors and retinal layers with near diffraction-limited resolution. The field-of-view (FOV) in most systems is constrained, typically $\leq 4^\circ$, which complicates the contextualization, interpretation, and utilization of the images in longitudinal studies and treatment trials. One solution is to stitch several overlapping images together into a montage with a larger FOV. Montaging facilitates the spatial alignment of AO data from repeated exams and also with data from other imaging modalities because more structural features (eg, vessels) are visible. Wide-field montages also provide a better opportunity to observe spatial patterns across the retina, whether due to disease or therapeutic response. Montaging is typically performed offline after the AO scan, either manually or automatically with registration software, and can be time-intensive due to repetitive cone patterns and the lack of unique features. With offline montaging, any coverage gaps in the montage and any insufficiencies or inefficiencies in the overlap between adjacent images cannot be corrected. Real-time montaging during the AO scan provides all the advantages of wide-field imaging plus the benefits of immediate feedback to the camera operator. With a live display of the montage building up during the scan, an operator can avoid gaps or recollect data before the patient has left. The operator can tailor the sequence of fixation target locations to the patient's individual fixation abilities, thereby reducing unnecessary overlap, improving efficiency, and shortening scan duration when possible. In this work, a real-time image montaging system is presented. For robust performance, image registration is performed by cross-correlation, implemented with frequency-domain phase modulations for efficiency, using the Fourier-Mellin transform to estimate rotation and translation parameters and parallel processing for speed. Results with a flood-illuminated AO camera demonstrate real-time performance with high registration accuracy and low latency.

IRB Status: Approved

Disclosures:

TRAVIS SMITH, PHD: Grant Support relationship with Fight for Sight

O245

EVALUATING THE FEASIBILITY OF FLOOD-ILLUMINATED ADAPTIVE OPTICS IN THE CLINICAL SETTING IN PATIENTS WITH INHERITED RETINAL DEGENERATION

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CASEY EYE INSTITUTE, OREGON HEALTH AND SCIENCE UNIVERSITY

Purpose: To evaluate the RTX1 flood-illuminated adaptive optics (AO) camera for its reliability and efficacy to provide structural diagnostic information in subjects with inherited retinal degenerative diseases. **Methods:** We used the RTX1 flood-illuminated adaptive optics camera (Imagine Eyes: Orsay, France) to image 139 subjects with suspected inherited retinal degeneration ranging in age from 7 to 88 years old. This included 63 patients with retinitis pigmentosa, 20 patients with Stargardt dystrophy, 21 patients with Usher syndrome, 11 patients with X-linked retinoschisis, 3 patients with choroideremia, and 3 patients with congenital stationary night blindness. For each subject, a series of $25.4^\circ \times 4^\circ$ retinal images were obtained in one or both eyes. Using i2k Retina (DualAlign LLC, Clifton Park, NY, USA), these images were combined to create a retinal montage spanning a $12^\circ \times 12^\circ$ field of the central macula. In a subset of higher quality images, cone counting was performed automatically by applying background subtraction and thresholding of local maxima in Matlab (MathWorks, Natick, MA, USA). All testing was approved by the OHSU IRB. **Results:** Image quality varied substantially between individual subjects and across pathologies. If the 9 central tiles and at least 20 of the 25 total images could be used to form a montage, then the imaging session was considered successful. The success rate was as follows: retinitis pigmentosa (73%), Usher Syndrome Type II (70%), Choroideremia (66%), Usher Syndrome Type I (45%), Stargardt Dystrophy (45%), CSNB (75%), XLRS (36%). The ability to see cone photoreceptors by AO usually correlated to the visualization of the ellipsoid zone by optical coherence tomography (OCT). In areas where the ellipsoid zone was not intact, regions of blur were seen on AO, which correlated with areas of hyperautofluoresence. Cone-like reflective spots were seen in areas of disrupted ellipsoid zone, but it was unclear if these truly represented cones or debris. **Conclusions:** Current flood illuminated adaptive optics technology is most effective when imaging subjects with good central vision, such as RP and Usher Type II patients. However, some low vision patients (such as CSNB) could be imaged. Patients with XLRS were among the most difficult to image.

IRB Status: Approved

Disclosures:

MARK PENNESI, MD, PHD: Travel Grant relationship with Imagine Eyes

OI02 – Innovations in Imaging Animal Models of Disease

O246

IMAGING RETINAL OXYGEN METABOLISM IN RODENT EYES

JI YI, Wenzhong Liu, Siyu Chen, Robert Linsenmeier, Hao Zhang

NORTHWESTERN UNIVERSITY

Oxygen metabolism is critical in maintaining retinal cell functionality, because the retina are highly metabolically active tissue type. The disorder of the oxygen metabolism could cause retinal cell degeneration in many retina-related disease, such as glaucoma, diabetic retinopathy, and age-related macular degeneration. Therefore, measuring retinal oxygen metabolism is invaluable in early detection of diseases and indication of disease progression. However, it is challenging to perform such a measurement in a non-invasive and label-free manner because measuring oxygen metabolism requires concurrent quantification of both blood flow and oxygen saturation rate (sO₂). Here we present an approach to tackle this challenge by using visible light optical coherence tomography (vis-OCT). OCT has become the gold standard in ophthalmology imaging, providing three dimensional morphological reconstruction with micron level resolutions. Using vis-OCT, we measured the absolute blood flow based on the phase variation provided by the moving blood cells. For the sO₂ measurement, we utilized the distinct absorption spectral contrast from deoxy- and oxyhemoglobin. The three dimensional imaging capability allows us to isolate the optical spectra from the retinal blood vessels so that we can eliminate the confounding signals from other retinal layers. We demonstrate the accuracy of the blood flow and sO₂ measurements *in vivo* in rodent eyes. With this technique, potential clinical and biological applications on human retinal diseases can be explored.

IRB Status: None

Disclosures:

JI YI, PHD: No financial relationships to disclose

O247

RETINAL VASCULAR OXYGEN TENSION IMAGING IN RODENT MODELS OF RETINOPATHY

MAHNAZ SHAHIDI

UNIVERSITY OF ILLINOIS AT CHICAGO

The purpose of the study is to report measurements of retinal vascular oxygen tension in rodent models of early experimental diabetes and oxygen-induced retinopathy. Optical section phosphorescence lifetime imaging was performed by projecting a laser line at an oblique angle on the retina following intravenous injection of an oxygen sensitive molecular probe. Optical sectioning allowed depth-resolved phosphorescence imaging and lifetime measurements. Retinal arterial and venous oxygen tension were calculated from phosphorescence lifetime. Retinal blood flow was measured by reflectance and fluorescent microsphere

imaging. Combined retinal oxygen tension and blood flow imaging provided measurements of oxygen delivery by the retinal circulation and global inner retinal oxygen metabolism in streptozotocin diabetic rats. Retinal arteriovenous oxygen tension difference was determined in oxygen-induced retinopathy mice. This imaging technique has the potential to broaden knowledge of disease pathophysiology, and thereby advance diagnostic and therapeutic procedures for retinal ischemic conditions.

This study was supported by the National Eye Institute grants, EY017918 and EY001792, and Research to Prevent Blindness, senior scientific investigator and unrestricted departmental awards.

IRB Status: Approved

Disclosures:

MAHNAZ SHAHIDI, PHD: Patents/Royalties relationship with UIC

O248

INTERACTION OF OCULAR TISSUES WITH ACOUSTIC RADIATION FORCE

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COLUMBIA UNIVERSITY MEDICAL CENTER, OPHTHALMOLOGY

Imaging technologies such as optical coherence tomography (OCT), ultrasound, confocal imaging, angiographic methods, autofluorescence imaging, and scanning Scheimpflug, among others, have been advancing in capabilities and sophistication, resulting in improved assessment of the eye from cornea to orbit. A common denominator of these technologies, however, is their limitation to passive visualization rather than provocative functional assessment of tissue characteristics. We are investigating the use of acoustic radiation force (ARF) as a means for characterizing ocular tissues. In conventional pulse/echo ultrasound imaging, the transducer does not emit energy 99.99% of the time. However, if the transducer emits continuously for periods on the order of 1-10 msec, sufficient energy can be absorbed by tissues to convert this into a compressive force. By interleaving pulse/echo sequences between bursts of ARF, force-induced tissue displacements can be observed with sub-micron precision. This constitutes a stress/strain relation that can provide information regarding tissue stiffness. We utilized this methodology to assess corneal and choroidal properties in the rabbit eye *in vivo*. Corneal studies were conducted with a focused, single-element 25 MHz transducer. We compared corneal stiffness before and up to 4 weeks following riboflavin crosslinking. At four weeks post-treatment, treated corneas were 1.3 times stiffer than pre-treatment, with no significant change in controls. Choroidal studies were carried out using a focused 20 MHz transducer. We detected displacement of up to 15 microns in orbital tissues and under 10 microns in the choroid. In proptosed eyes with elevated intraocular pressure, unusual transient (<1 sec) increases in choroidal backscatter were noted following ARF, suggestive of an ARF-induced transient increase in local perfusion. We are currently planning studies utilizing OCT to document ARF-induced effects (including vibration) with higher spatial and temporal resolution.

IRB Status: Approved

Disclosures:

RONALD SILVERMAN, PHD: No financial relationships to disclose

O249

BRILLOUIN MICROSCOPY FOR CORNEAL IMAGING

SEOK-HYUN (ANDY) YUN

HARVARD MEDICAL SCHOOL

We have previously developed Brillouin microscopy to measure the elastic modulus of the crystalline lens and the cornea with spatial resolution. In this talk, I will present optical instrumentation, results of human pilot studies, and potential of this technique for translation into the clinic.

IRB Status: Approved

Disclosures:

SEOK-HYUN (ANDY) YUN, MD: No financial relationships to disclose

OI03 – Imaging Structure and Function in Glaucoma

O250

IN VIVO IMAGING OF THE 3D LAMINA CRIBROSA MICROARCHITECTURE IN NORMAL AND GLAUCOMATOUS HUMAN EYES

GADI WOLLSTEIN, Bo Wang, Zach Nadler, Hiroshi Ishikawa, Richard Bilonick, Larry Kagemann, Ian Sigal, Joel Schuman

UNIVERSITY OF PITTSBURGH, DEPARTMENT OF OPHTHALMOLOGY

The lamina cribrosa (LC) is a prime location of glaucomatous damage. Current studies looking at in vivo LC changes in glaucoma have been primarily limited to analyzing macro-architecture features. However, the 3D micro-architecture of the lamina, the collagenous support structure, and the axons passing through them have not been analyzed in vivo. In this study we compared LC 3D micro-architecture between healthy and glaucomatous eyes in vivo by using optical coherence tomography (OCT). Sixty-eight eyes (19 healthy and 49 glaucomatous) from 47 subjects were scanned in a 3.5 x 3.5 x 3.64-mm volume at the optic nerve head by using swept-source OCT. The LC micro-architecture parameters were measured on the visible LC by an automated segmentation algorithm of our own design. This segmentation method has been shown to provide reproducible LC measurements. The LC parameters were compared to diagnosis and visual field mean deviation (VF MD) by using a linear mixed effects model accounting for age. The average VF MD for the healthy and glaucomatous eyes was -0.50 ± 0.80 dB and -7.84 ± 8.75 dB, respectively. Beam thickness to pore diameter ratio ($P = 0.04$) and pore diameter standard deviation ($P < 0.01$) were increased in glaucomatous eyes. With worse MD, beam thickness to pore diameter ratio ($P < 0.01$), pore diameter standard deviation ($P = 0.05$), and beam thickness ($P < 0.01$) showed

a statistically significant increase while pore diameter ($P = 0.02$) showed a significant decrease. No significant interactions were detected between any of the parameters and age (all $P > 0.05$). Taken together, glaucomatous micro-architecture changes in the LC, detected by OCT analysis, reflect beams remodeling and axonal loss leading to reduction in pore size and increased pore size variability.

IRB Status: Approved

Disclosures:

GADI WOLLSTEIN, MD: No financial relationships to disclose

O251

HIGH-RESOLUTION IN VIVO CHARACTERIZATION OF LAMINA CRIBROSA MICROARCHITECTURE AND OPTIC NERVE HEAD CHANGES IN EARLY GLAUCOMA

JASON PORTER¹, Kevin Ivers¹, Nripun Sredar², Nimesh Patel¹, Lakshmi Rajagopalan¹, Hope Queener¹, Ronald Harwerth¹, George Zouridakis²

UNIVERSITY OF HOUSTON, COLLEGE OF OPTOMETRY¹; UNIVERSITY OF HOUSTON, DEPARTMENT OF COMPUTER SCIENCE²

Substantial evidence suggests that retinal ganglion cell axons are initially damaged at the level of the lamina cribrosa in glaucoma. We longitudinally examined changes in the optic nerve head (ONH), anterior lamina cribrosa surface (ALCS) microarchitecture and retinal nerve fiber layer thickness (RNFLT) to better understand the sequence of structural changes in early experimental glaucoma (EG). ONH parameters (mean ALCS depth [ALCSD], mean minimum rim width [MRW], mean radius of curvature of the ALCS) and RNFLT were calculated from spectral domain optical coherence tomography images of the ONH acquired before and approximately every 2 weeks after inducing unilateral EG in 7 rhesus monkeys. Adaptive optics scanning laser ophthalmoscope (AOSLO) images of ALCS microarchitecture were acquired at all time-points and used to calculate ALCS pore geometry globally and locally (in 60° sectors and central and peripheral regions). Changes in predominant ALCS beam orientation were also calculated at corresponding regions of interest in AOSLO images from 4 monkeys using principal components analysis. The first structural parameter to significantly change from baseline in 6 of 7 EG eyes was mean ALCSD. A significant decrease in mean MRW also occurred first in 4 EG eyes while a significant change in ALCS pore geometry occurred first or second in 4 EG eyes. The first significant changes in predominant ALCS beam orientation occurred simultaneously with the first change in ALCS pore geometry. RNFLT was the last structural parameter to significantly change. The fact that local changes in ALCS pore geometry were measured before a global change in the same parameter in some EG eyes suggests that local analyses can play a role in detecting earlier changes in laminar microarchitecture and provide insights on biomechanical remodeling of the lamina in glaucoma.

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IRB Status: Approved

Disclosures:

JASON PORTER, PHD: No financial relationships to disclose

O252

ASSESSING FOCAL RETINAL GANGLION CELL FUNCTION IN GLAUCOMA USING THE ELECTRORETINOGRAM

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STATE UNIVERSITY OF NEW YORK, COLLEGE OF OPTOMETRY¹;
UNIVERSITY OF HOUSTON²; VA MEDICAL CENTER, INDIANAPOLIS³

The purpose of these experiments was to determine how well the multifocal Photopic Negative Response (mfPhNR), a reflection of local retinal ganglion cell function measured using the electroretinogram, correlates with corresponding retinal structural measures using Optical Coherence Tomography and visual field sensitivity measures using perimetry in primary open angle glaucoma patients and nonhuman primates (NHP) with experimental glaucoma. The visual stimuli consisted of black and white array of 7 or 19 unstretched hexagons subtending 12 or 7 degrees respectively. The stimulus sequence was sufficiently slowed that there was a minimum of a 400 millisecond interval between successive flashes at the same location. Significant reductions in mfPhNR amplitudes were seen in glaucomatous eyes relative to control eyes in humans as well as NHPs ($P < 0.0001$ and $P < 0.005$). Local mfPhNR amplitudes were significantly correlated with corresponding sectoral Retinal Nerve Fiber Layer thickness; $R = 0.45$ ($P < 0.0001$) and > 0.78 ($P < 0.0001$) for humans and NHP. Similarly good correlations were seen between mfPhNR and retinal ganglion cell/inner plexiform layer thickness that was measured only in NHPs; $R > 0.77$ ($P < 0.0001$). Longitudinal measurements in NHPs demonstrated that mfPhNR amplitude changes preceded parallel changes in corresponding structural measures. Local mfPhNR amplitudes were correlated moderately with local perimetric visual field sensitivity; $R > 0.42$ ($P < 0.0001$) and 0.41 ($P < 0.001$) for humans and NHP. mfPhNR amplitude reduction in the the macular region preceded perimetric visual field sensitivity changes in glaucoma patients. These findings indicate that the mfPhNR can be used as an additional tool to detect and monitor functional changes in glaucomatous eyes. The reasons why mfPhNR amplitude changes precede clinical measures of retinal ganglion cell structure and function in glaucomatous eyes need further investigation.

IRB Status: Approved

Disclosures:

SURESH VISWANATHAN, PHD: No financial relationships to disclose

O253

ASSESSING NEURONAL DAMAGE CAUSED BY GLAUCOMA

RONALD HARWERTH, Nimesh Patel

UNIVERSITY OF HOUSTON

The glaucomas are a group of relatively common optic neuropathies, in which the pathological loss of retinal ganglion cells causes progressive structural alterations in the retina and optic nerve head, with associated functional losses in visual sensitivity. The purpose of this presentation is to describe relationships between structure and function in glaucoma as gauged by reduced visual sensitivity, loss of retinal ganglion cells (RGCs), and thinning of the retinal nerve fiber layer (RNFL) and neuroretinal rim (NRR). Two groups of subjects were studied; 1) macaque monkeys with experimental glaucoma and 2) clinical patients with glaucoma. In both groups, standard automated perimetry (SAP) was used to measure functional defects and spectral domain, optical coherence tomography (SD OCT), incorporating individualized transverse scaling and custom segmentation, was used to measure structural defects. The results of the studies of experimental glaucoma showed that the onset and degree of structural defects were correlated to the cumulative intraocular pressure (mm HG – days) while functional defects were not. The assessment of temporal relations were consistent across macaque and human subjects, with the earliest signs of glaucomatous damage from thinning of the NRR, either total volume or minimum width, with subsequent loss of RNFL followed by SAP. The primary limit for the identification of glaucomatous neuropathy and the detection of progression was measurement variability, which is considerably smaller for SD OCT (coefficient of variability (CV) of 2 – 18%) compared to subjective SAP measures (CV of 34 – 48%). In conclusion, these results provide support for the use of experimental glaucoma in macaque monkey as a model for investigations of structure-function that are directly applicable to clinical patients. The findings provide support for the use of multiple OCT derived scaled morphological measures for the diagnosis and management of primary open angle glaucoma.

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Disclosures:

RONALD HARWERTH, OD, PHD: No financial relationships to disclose

O254

QUANTUM DOTS TRAVEL FROM THE ANTERIOR CHAMBER OF THE EYE INTO THE OPTIC NERVE AND THIS IS ENHANCED BY LATANOPROST

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LABORATORY MEDICINE AND PATHOBIOLOGY, UNIVERSITY OF TORONTO, ST. MICHAEL'S HOSPITAL

The relationship between aqueous humor and tissues of the back of the eye, particularly the optic nerve, is poorly understood. Latanoprost is a prostaglandin $F_{2\alpha}$ analog that stimulates drainage from the eye to treat glaucoma. We hypothesize that communication between the anterior chamber and optic nerve exists, and is enhanced by latanoprost. To test this, a fluorescent nanoparticle tracer, Quantum Dot 655 (QD; Invitrogen, OR, USA), was injected into the eye of male 129SVE mice

(n=24) under general isoflurane anesthesia. 3 μ L of QD was injected into the left anterior chamber of mice that had received topical application of 3 drops of latanoprost (Xalatan, Pfizer, QC, Canada) (n=12) or artificial tear (n=12) to both eyes the day before and 1 hour prior to tracer injection. Mice were sacrificed 6 hours after injection and enucleated left orbits were fixed in paraformaldehyde then frozen sectioned (140 μ m). Sections were analyzed with fluorescence microscopy (Olympus BX-50, PA, USA) to determine how far QD traveled down the optic nerve. Mann-Whitney U test was used for statistical comparison. Results show that 6 hours following intracameral injection, QD was found in the ipsilateral optic nerve of both untreated (7/12) and latanoprost-treated groups (8/12). QDs in latanoprost-treated mice traveled further posteriorly compared with untreated mice ($718 \pm 144 \mu\text{m}$ vs. $218 \pm 62 \mu\text{m}$ (mean \pm SE); $p < 0.02$). We conclude that quantum dots move from the anterior chamber into the optic nerve and this is enhanced by latanoprost. This finding is relevant to understanding pathways of drugs injected into the eye, and also drug delivery for back of the eye disease.

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IRB Status: Approved

Disclosures:

EMILY MATHIEU, BSC: No financial relationships to disclose

OI04 – New Approaches to Imaging Retinal and Choroidal Vasculature

O255

EFFECT OF ANTI-VEGF THERAPY ON CHOROIDAL THICKNESS IN DIABETIC MACULAR EDEMA

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DUKE UNIVERSITY MEDICAL CENTER, OPHTHALMOLOGY¹; DUKE UNIVERSITY, BIOMEDICAL ENGINEERING²

Purpose: To determine the effect of anti-vascular endothelial growth factor (VEGF) therapy on choroidal thickness in eyes with diabetic macular edema (DME)

Design: A retrospective, cohort analysis

Participants: 59 eyes of 59 patients with DME without prior anti-VEGF therapy

Methods: Choroidal thickness (CT) was measured using semi-automated segmentation of enhanced-depth imaging optical coherence tomography (EDI-OCT) images at 0.5 mm intervals from 2.5 mm nasal to 2.5 mm temporal to the fovea. Best-corrected visual acuity (BCVA) and central foveal thickness (CFT) were analyzed to evaluate the association of CT with functional and anatomical outcomes.

Main Outcome Measures: Changes in CT with and without anti-VEGF treatment over 6 months

Results: Of the 59 eyes with DME, 26 eyes were observed without treatment, while 33 underwent intravitreal anti-VEGF therapy (mean number of injections = 2.73) over 6 months. In untreated eyes, there was no significant change in BCVA ($p = 0.098$), CFT ($p = 0.472$), or CT at all measurements along the macula ($p = 0.057$ at the fovea). In eyes treated with anti-VEGF injections, CT significantly decreased at the fovea (246.6 mm to 224.8 mm; $p < 0.001$) and at 0.5 mm nasal (240.9 mm to 221.9 mm; $p = 0.002$) and 0.5 mm temporal (249.3 mm to 224.8 mm; $p = 0.011$) to the fovea. The decrease in subfoveal CT after anti-VEGF treatment was not associated with the cumulative number of anti-VEGF injections ($R^2 = 0.031$, $p = 0.327$), or to changes in BCVA ($R^2 = 0.017$; $p = 0.470$) or CFT ($R^2 = 0.040$; $p = 0.263$).

Conclusions: Central CT decreases after anti-VEGF therapy for DME after 6 months, and may not be associated with functional or anatomical outcomes in eyes with DME.

IRB Status: Approved

Disclosures:

GLENN YIU, MD, PHD: No financial relationships to disclose

O256

PROGRESS ON OCT BASED IMAGING OF CHORIOCAPILLARIS IN THE LIVING HUMAN EYE

ROBERT ZAWADZKI

UNIVERSITY OF CALIFORNIA DAVIS

Choriocapillaris (CC), one of the vascular layers of choroid that is immediately adjacent to Bruch's membrane, is responsible for supplying nutrients to the outer retinal layers (Retinal Pigment Epithelium (RPE) and photoreceptor complex) as well as for removal of metabolic waste from the same region. To achieve that, CC stays in dynamic symbiosis with RPE and thus plays a critical role in normal retinal function. Due to the difficulty in studding CC structure and function in vivo, the role of CC in initiation and/or progression of many eye diseases remains to be determined.

Historically the main challenges associated with imaging CC in vivo included its location, below light scattering RPE, and fenestration of vessels making these structures not visible with fluorescein and indocyanine green (ICG) angiography. Therefore, current knowledge of CC structure is based mostly on multiple histological studies, making the ability to image CC *in vivo* critical for better understanding of pathophysiology of many retinal diseases that affect Photoreceptor-RPE-Bruch's Membrane-CC complex.

Thanks to the recent progress in flow contrast based - Optical coherence tomography (OCT) methods, several groups have shown contrast agent-free depth-resolved vascular perfusion maps of retina and choroid including first reports of visualizations of CC vasculature in the living human eye.

In this talk, I will review OCT methods currently used for CC imaging with main focus on phase variance OCT (pv-OCT)

method, including example images of Retinal and Choroidal vasculature. Challenges associated with data acquisition and processing for these methods will be discussed as well.

IRB Status: Approved

Disclosures:

ROBERT ZAWADZKI, PHD: No financial relationships to disclose

0257

PHOTOACOUSTIC VASCULAR IMAGING IN THE EYE

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Purpose: We want to develop non-invasive optical imaging methods that can quantify retinal metabolic rate of oxygen (MRO₂) using combined photoacoustic imaging and optical coherence tomography.

Methods: We combined photoacoustic ophthalmoscopy (PAOM) and Doppler OCT to achieve this goal, where PAOM measured retinal hemoglobin oxygen saturation (sO₂) and Doppler OCT measured retinal flow. In PAOM, nanosecond laser pulses illuminate the retina at a 20-kHz pulse repetition rate and a high-sensitivity small-footprint ultrasonic transducer (center frequency: 35 MHz; bandwidth: 50%) detects the ultrasonic waves generated from retina as a result of optical absorption. To measure retinal sO₂, a circular trajectory was scanned around the optic disk and three optical wavelengths (570 nm, 578 nm, and 588 nm) were used sequentially. Based on the molecular extinction coefficients differences between oxy-hemoglobin and deoxy-hemoglobin at the three optical wavelengths, sO₂ in each retinal vessel scanned by the circular trajectory was calculated. The PAOM was also integrated with an SD-OCT, which provided optical alignment prior to PAOM imaging and complementary imaging contrast to PAOM. In Doppler OCT, we performed double-circular-trajectory scan around the optical disk to obtain the absolute retinal blood flow. As a result, the total retinal MRO₂ was calculated.

Results: Through circular scanning around the optic disk, multi-wavelength PAOM acquired the sO₂ levels in all the major retinal vessels and, therefore, retinal veins and arteries were separated according to the measured sO₂ values, which agreed with other literature data. Doppler OCT scanned the same circular trajectory as PAOM and the blood flow velocity in every retinal vessel imaged by PAOM was obtained. As a result, retinal arteries and veins were also separated based on the fact that venous bloods flow inward and arterial bloods flow outward. The two independently achieved arterial-venous separations matched in every single vessel. We imaged several Sprague Dawley rats (body weight: ~500 g) and the results were consistent. We also calculated the total blood flow volumes in all the retinal arteries (21.8 μ L/min) and veins (23.6 μ L/min) and the two volumes agreed with each by a 7.6% difference. After acquiring the total hemoglobin concentration in rat blood samples, the MRO₂ was calculated to be 414 \pm 94 nL/min.

Conclusions: We developed a multimodal method, combining functional PAOM with Doppler OCT, to image both the

anatomic and functional parameters of the retinal vascular system. This novel method holds promise in both fundamental study and clinical diagnosis of retinal diseases, such as diabetic retinopathy, which manifest early pathological alterations in oxygenation and metabolism.

IRB Status: NONE

Disclosures:

HAO ZHANG, PHD: No financial relationships to disclose

0258

VASCULAR IMAGING IN DIABETIC RETINOPATHY

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Several key structures of retinal and choroidal blood vessels are altered in diabetic retinopathy. There are two general types of damage known from histology. When blood flow is compromised, ischemia and the failure to provide nutrients and wound healing lead to neural damage. When the retinal vascular system initiates a wound healing response, there is not repair damage to the retina, but instead hyper-perfusion, endothelial cell proliferation and migration, and neovascularization. Both types of vascular damage, the ischemic and the exudative, can lead to sight threatening retinopathy. Macular edema is a common cause of vision loss and can go undetected for years. Both inner and outer blood retinal barriers can be associated with macular edema. We have found both types of abnormal vascular change, prior to the onset of clinically detectable diabetic retinopathy. This problem is made worse for underserved patients who are often not examined promptly after diagnosis of diabetes, or for about 25% of diabetics who are undiagnosed. We present imaging technologies that improve the visualization of small but key changes in the development of diabetic retinopathy. Using the microscopic views provided by Adaptive Optics Scanning Laser Ophthalmoscopy, we visualize capillary sprouts, abnormal vessel walls, non-perfused capillaries, disturbed blood flow through microaneurysms, and other pathological changes. Using Spectral Domain Optical Coherence Tomography and novel image processing techniques, we demonstrate dilated and hyper-reflective retinal vessels of diabetic patients as compared with controls, sometimes occurring without measurable retinal thickening. Using low cost devices, such as the Laser Scanning Digital Camera or Digital Light Ophthalmoscope, we can acquire high contrast digital retinal images that document clinically visible features of diabetic retinopathy. The potentially high contrast of these new low cost devices may provide a link between the novel findings of highly magnified devices and the present understanding of diabetic retinopathy.

IRB Status: Approved

Disclosures:

ANN ELSNER, PHD: Equity Owner relationship with Aeon Imaging, LLC; Patents/Royalties relationship with Aeon Imaging, LLC; Grant Support relationship with Aeon Imaging, LLC

O259

TRABECULAR MESHWORK STRESS-STRAIN RESPONSE IN LIVING VERSUS DONOR EYES: A PILOT STUDY

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We recently demonstrated that a trabecular meshwork (TM) strain response can be measured in living eyes.(1) Combining Schlemm's Canal (SC) cross-sectional area (CSA) and TM thickness (Tmt) as a surrogate measure of TM strain (expansion), TM encroachment into SC is quantifiable during large acute intraocular pressure (IOP) elevation¹. In living eyes undergoing SC collapse, TM thickness changes little, suggesting that the TM acts as a membrane under tension, possibly due to the influence of the ciliary body (CB).(2) This presentation will examine TM strain in response to small IOP elevations, and the role the CB in preventing SC collapse in living eyes. This is accomplished by examining the temporal limbus by spectral-domain optical coherence tomography (Cirrus HD-OCT, Zeiss, USA) at baseline and during IOP elevations with 5 and 10g of pressure applied to the sclera. SC-CSA, SC inner to outer wall distance (IOD), and Tmt are compared with measurements by Van Buskirk et al in perfused cadaveric eyes. (3) This protocol is repeated in a subset of subjects with and without suppression of CB activity by 1 drop of tropicamide. The findings will suggest that, when presented with small acute IOP elevations, the active CB stabilizes IOD and SC-CSA. CB suppression allows SC to narrow. When presented with small acute IOP elevations, the living eye appears to regulate outflow pathway patency, presenting with little or no observable tissue strain, compared to donor eyes in a perfusion model. Suppression of CB activity inhibits the eye's ability to regulate SC-CSA and IOD, allowing a response more similar to that of donor tissues.

1. Kagemann L, et al. IOP Elevation Reduces SC-CSA. IOVS 2014
2. Kagemann L, et al. Stiffness of the Trabecular Meshwork In Living Eyes. ARVO 2014
3. Van Buskirk EM. Anatomic correlates of changing aqueous outflow facility. IOVS 1982

IRB Status: Approved

Disclosures:

LARRY KAGEMANN, PHD: No financial relationships to disclose

O260

TOWARDS IMPROVED GLAUCOMA MANAGEMENT: MAPPING OPTIC NERVE HEAD BIOMECHANICS USING SPECTRAL-DOMAIN OCT, MICRO-OCT, AND ADAPTIVE OPTICS

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NATIONAL UNIVERSITY OF SINGAPORE, BIOMEDICAL ENGINEERING¹; NANYANG TECHNOLOGICAL UNIVERSITY, SCHOOL OF ELECTRICAL AND ELECTRONIC ENGINEERING²; MOORFIELDS EYE HOSPITAL, NIHR BIOMEDICAL RESEARCH CENTRE³

The optic nerve head (ONH) is the major site of damage in glaucoma. Previous studies have suggested that measurements of ONH strains, as induced by intraocular pressure (IOP) may provide a crucial understanding of the underlying mechanisms involved in glaucoma, and may lead to improved diagnosis. Here, we present engineering tools to map IOP-induced ONH strains using 3 imaging modalities.

Using spectral-domain OCT, eight ONHs from glaucoma patients were imaged before and after therapeutic IOP reduction. In each low-IOP volume, the lamina cribrosa (LC) was manually segmented. For each ONH, an improved tracking algorithm was applied to both OCT volumes to extract IOP-induced displacements and strains. Tracking improvements included: 1) speckle-noise reduction; 2) sparse-to-dense grid tracking; 3) self-adaptive optimization; 4) robust displacement smoothing; and 5) speed optimization. Using artificially deformed OCT volumes of known deformation, tracking was found robust and could extract displacements with an error of 0.51 μm . LC strain in the glaucoma eyes ranged from 2.6% to 15.1% and was higher than that measured in 1 normal control (1.7%).

Using adaptive optics, the LC of 2 normal patients was continuously imaged en-face for 4 seconds. All movies were processed using Eulerian Video Magnification to exaggerate deformations. Our preliminary assessment indicates that LC deformations synchronize with the heart rhythm, suggesting that the ocular pulse is responsible for such deformations.

Using micro-OCT, we demonstrated the ability to image the LC pores and beams at 1-micron resolution in enucleated porcine eyes. Such images can be used for strain mapping.

We present here engineering tools that can map LC deformations using 3 imaging modalities. Our ultimate goal is to verify whether an association exists between LC strains and loss of visual function in glaucoma.

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IRB Status: Approved

Disclosures:

MICHAEL JA. GIRARD, PHD: No financial relationships to disclose

VASCULAR MAPPING WITH AN AOSLO

STEPHEN BURNS, Gang Huang, Alberto deCastro, Lucie Sawides, Ting Luo, Thomas Gast

INDIANA UNIVERSITY

Modern adaptive optics scanning laser ophthalmoscopes (AOSLO) allow the researcher to generate superb images of the human retinal vasculature. They also allow us to image the motion of red blood cells as they flow through the vascular system. This has allowed us to map the entire vascular tree from artery to capillary to vein. The disadvantage is that AOSLO systems typically need a large number of video frames to generate a vascular map, this can translate into long recording times. We investigated different approaches for improving this process. We imaged the retina in given regions of both patients (diabetics, and hypertensives and normal controls). We use aligned and offset aperture confocal imaging, in a normal single channel imaging approach or a new dual temporal offset imaging technique (Huang et al ARVO 2014). The single channel images were collected at 30 Hz and the presence of blood flow is computed by the temporal variation, for our techniques using from 20 to 300 frames. In temporal offset imaging, a single scan system, is used to image the retina with two closely related wavelengths (785 and 810 nm) that enter the eye at slightly different angles (~0.01 deg). Because we use a scanning system this means that a given point on the retina will be scanned at time differentials between 0.8 and 2.2 msec. Because the major change occurring at these very short time scales arise from blood flow, difference maps can show movements of single cells and we use the differentiate intact vessels. All three methods can provide vascular maps although the signal to background varied for a similar frame count with the offset imaging being better. The combination of offset pinhole imaging with temporal offset imaging could generate the maps with the shortest total recording time.

IRB Status: Approved

Disclosures:

STEPHEN BURNS, PHD: Equity Owner relationship with AEON imaging

CHOROIDAL OCT

WOLFGANG DREXLER, Marieh Esmaeelpour

MEDICAL UNIVERSITY OF VIENNA

To date, commercial and scientific clinical ophthalmic OCT has mainly been performed in the 800nm region with only limited penetration beyond the retina. For that reason, *in vivo* OCT imaging in the 1060nm wavelength region has been initiated for significant better choroidal visualization. Several generations of spectral domain as well as swept source based OCT systems have been developed with scanning speeds from

15.000 to 100.000 A-scans/second and axial resolutions of 6-8 μm . Recently a simultaneous 800/1060nm eye-tracked spectral domain OCT system based on a commercial OCT machine has been developed for speckle free dual wavelength retina/choroidal OCT. Three-dimensional 1060nm OCT improves imaging performance in eyes with turbid ocular media (corneal haze or cataract) and enables significantly better visualization of choroidal morphology independent of retinal pigmentation. Two-dimensional choroidal thickness maps indicate a thicker choroid in hyperopes with more variation among than as compared to a thinner choroid in myopes with less variation among them compared to emmetropes. Central choroid is thinner in all type 2 diabetic eyes regardless of disease stage. The choroidal thinning may exceed the magnitude of possible choriocapillaris atrophy. Choroidal thickness is decreased in diabetes type 1, independent of the absence of pathology and of diabetic disease duration. In eyes with pathology, averaged choroidal thickness maps showed an extension of the thinning area matching retinal lesions and suggesting its involvement on onset or progression of disease. Retinal thickness is reduced in early AMD, but choroidal thickness seems to be unaffected. Therapy seems to be more effective in wet AMD patients with thinner choroid.

IRB Status: International

Disclosures:

WOLFGANG DREXLER, PHD: No financial relationships to disclose

NEW FINDINGS ON SCLERAL COLLAGEN ARCHITECTURE USING MICRON-SCALE FIBER ORIENTATION ANALYSIS

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The collagen fibers of the posterior pole are central to the physiology and pathophysiology of the eye. Our goal was to map the architecture of these fibers, identify major organizational components and measure the distribution of collagen fiber crimp. Three eyes from 3 donors, and 8 eyes from 4 sheep were fixed at IOP=5mmHg, and the posterior poles cryosectioned coronally. An average of 75 sections per eye (range 42-154) from extraocular optic nerve to the vitreous were imaged with a microscope (4.1 μm /pixel) and local collagen fiber orientation was determined using a technique described elsewhere (IOVS 54:E-abstract 65). Mean and SD of fiber orientation were measured in 674 areas of three types: sclera immediately adjacent to the canal, innermost sclera and random sclera elsewhere. Sections through the sheep lamina cribrosa were selected and imaged again (0.80 μm /pixel). In these, crimp period was measured manually in regions proximal (<900 μm) and distal (1400-3000 μm) to the canal.

Linear mixed effect models were fit to test if crimp period, mean angle or angle variations were significantly predicted by region accounting for correlations within eye and individual. Distal crimp period ($39.4 \pm 6.1 \mu\text{m}$) was significantly ($p < 0.001$) larger than proximal ($65.1 \pm 18.8 \mu\text{m}$). Area mean orientations were significantly different between all regions ($p < 0.0001$), with strong alignments in the radial and innermost regions and isotropic orientations in the random region. We have identified a previously unreported subpopulation of collagen

fibers in the innermost sclera highly aligned radially from the canal. We estimated these to be $30\text{-}90\mu\text{m}$ thick in human and $60\text{-}120\mu\text{m}$ thick in sheep. It is likely that these structures and the 50% larger crimp distal vs. proximal to the canal confer distinct biomechanical properties to these regions.

IRB Status: International

Disclosures:

IAN SIGAL: No financial relationships to disclose

RPE Biology and Pathology

RP01 – RPE Replacement and Tissue Engineering

O264

TISSUE ENGINEERING CAN PAVE THE WAY FOR RPE FROM THE LAB INTO SUBRETINAL SPACE

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UNIVERSITY EYE HOSPITAL BONN

Replacement of the RPE monolayer may represent a curative treatment option for patients with age-related and/or Stargardt's macular degeneration. Suspension RPE transplantation and RPE/choroid patch translocation were attempted in the past, but faced several biological and technical challenges. Several RPE stem cell sources are currently under consideration in translational projects within or towards phase 1/2 clinical trials for RPE replacement.

The fundamental concept of tissue engineering is the use of cell carriers and/or encapsulation optionally combined with bioactive molecules by which (cell) transplants are engineered in the laboratory, then packaged, delivered and integrated at their eventual target site. When applied to RPE transplantation, here a RPE population of choice is pre-cultured on a prosthetic Bruch's membrane into a functional monolayer, optionally encapsulated into a biodegradable hydrogel and followed by transplantation of these RPE/scaffold complexes underneath the retina. Such "packaging" may enable facile precise delivery and ensure subsequent graft function, when compared to prior art.

This introductory lecture will briefly explore common tissue engineering concepts with relevance to RPE replacement, illustrated with some own adaptations.

IRB Status: Approved

Disclosures:

BORIS STANZEL, MD, FEBO: No financial relationships to disclose

O265

STRATEGIES FOR CELLULAR THERAPY TO TREAT RETINAL DEGENERATION

DENNIS CLEGG, Sherry Hikita, Qirui Hu, Lincoln Johnson, Roxanne Croze, Tracy Clevenger, Lyndsay Leach, Britney Pennington

UC SANTA BARBARA

Age-related macular degeneration (AMD) is one of the leading causes of blindness, and there are few treatment options for the dry form. As the disease progresses, retinal pigmented epithelial (RPE) cells in the macula lose function or die, and as a result, photoreceptors are lost. One promising option is to develop a

cellular therapy using RPE derived from pluripotent stem cells. Both hESC-RPE and iPS-RPE have been shown to be similar but not identical to fetal human RPE, and one clinical trial is underway that employs a bolus injection of hESC-RPE cells. Another strategy is to implant differentiated, polarized monolayers of hESC-RPE or iPS-RPE on a scaffold, whereby cells are provided with a supportive substrate. We describe the recent strategies to develop such a regenerative patch for the treatment of dry AMD.

IRB Status: None

Disclosures:

DENNIS CLEGG, PHD: Equity Owner relationship with Regenerative Patch Technologies

O266

CELL CARRIER SUBSTRATES FOR RPE REPLACEMENT

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UNIVERSITY OF LIVERPOOL

Replacing Retinal pigment epithelial (RPE) cells offer a theoretical treatment strategy for a wide variety of retinal diseases including "dry" age-related macular degeneration and the RPE dystrophies. The proof of concept that vision could be improved was that by placing a healthy neuroretina onto a different, healthy, underlying differentiated RPE layer is demonstrated in patch graft transplantations. Unfortunately the surgical procedure to relocate the neuroretina is both complex and is hampered by postoperative complications. Cell replacement therapies are therefore being researched. The choice of which cells to transplant is complicated by factors such as the ease of collection of an adequate sample, rejection following implantation, the age of the cells and ethical and regulatory issues. Also many studies have focused on using cell suspensions and have had disappointing outcomes largely due to the lack of control over cellular differentiation, incomplete attachment onto a diseased Bruch's membrane and subsequent integration into the existing RPE monolayer. The use of cell carrier substrates will enable differentiated monolayers to be transplanted at the outset. We have investigated the use of polyurethanes using an autologous approach with RPE and Iris pigment epithelial cells (IPE). Both IPE and RPE can be successfully cultured on porous PUs. The cells formed a distinct monolayer expressing ZO-1 tight junctions and were able to demonstrate functionality with their ability to phagocytose photoreceptor outer segments. The ability to form functioning monolayers alongside the porosity and mechanical properties of PUs make them suitable cell carrier substrates.

Funded by the Macular Disease Society, UK.

IRB Status: None

Disclosures:

CARL SHERIDAN, PHD: No financial relationships to disclose

DEVELOPMENT OF A RPE/CHOROIDAL STROMAL SUBSTITUTE MODEL USING THE SELF-ASSEMBLY APPROACH OF TISSUE ENGINEERING

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Véronique Beaulieu-Leclerc, Solange Landreville

CHU DE QUÉBEC RESEARCH CENTER - HÔPITAL ST-SACREMENT

The purpose of this study was to develop a choroidal stromal substitute on which retinal pigment epithelial (RPE) cells can be seeded in order to develop an in vitro model. Human choroids were incubated in dispase to isolate RPE cells, then in collagenase to dissociate the choroidal cells. Vascular endothelial cells were isolated using CD31-coated magnetic beads. The remaining cells were plated in growth media optimized for the culture of melanocytes or fibroblasts. To reconstruct the choroidal stroma, fibroblasts were cultured in the presence of serum and ascorbic acid to promote extracellular matrix (ECM) assembly. The technique used for cell isolation yielded pure cultures of RPE, fibroblasts, melanocytes and vascular endothelial cells, as assessed using immunostainings (K8/18, vimentin, HMB45, CD31). The stromal substitutes engineered using the self-assembly approach were composed of collagens, proteoglycans and other ECM proteins. Protein expression was confirmed using immunostaining. Endothelial cells spontaneously assembled into tubular structures and vascular networks when cocultured within the fibroblast-containing ECM sheets. Interestingly, when RPE cells were added, the vascular network covered a higher percentage of the area and contained a larger number of ramifications. To achieve a complete choroidal substitute model, we also assessed their suitability to culture choroidal melanocytes. The seeded melanocytes adhered and survived on the stromal substitutes as confirmed by the presence of pigmented HMB45-positive cells with a dendritic morphology. In conclusion, this study shows that the self-assembly approach of tissue engineering can be used to reconstruct a choroid-like tissue using native cells. This model represents a unique tool to better understand the crosstalk between the different choroidal cell types and cell-ECM interactions, and could potentially be used as a carrier for RPE replacement therapies.

Support: FRQS, ThéCell Network, Vision Health Research Network, HSS-HEJ Foundation.

IRB Status: International

Disclosures:

STEPHANIE PROULX, PHD: No financial relationships to disclose

SURGICAL ASPECTS OF STEM CELL-DERIVED RETINAL PIGMENT TRANSPLANTATION IN ANIMALS

MICHAEL KOSS

OPHTHALMOLOGY, UNIVERSITY OF HEIDELBERG

To evaluate the benefits and risks of a subretinal transplantation of human embryonic stem cell derived RPE cells covered

on a non-degradable, biocompatible parylene membrane, specific surgical aspects are important. We will present key findings from our experience from nude athymic rats, the RCS rats, rabbits and yucatan mini-pigs. The latter animal model was selected to project specific risks from a 3 port pars plana vitrectomy, that would be realistic for a subretinal transplantation in human eyes.

IRB Status: Approved

Disclosures:

MICHAEL KOSS, MD, PHD: No financial relationships to disclose

RP02 – Phagosomes, Endosomes, and Lipofuscin in the RPE

REDUCE, REUSE, RECYCLE: MECHANISMS OF CELLULAR CLEARANCE IN THE RPE

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UNIVERSITY OF WISCONSIN-MADISON, OPHTHALMOLOGY AND VISUAL SCIENCES

The retinal pigment epithelium (RPE) performs numerous functions essential for photoreceptor health and vision. Central to these functions is a highly dynamic endo-lysosomal network that communicates with the phagocytic and autophagic pathways to maintain a clean environment in the outer retina. The post-mitotic nature of the RPE places additional pressure on its endo-lysosomal and autophagy systems because they have to perform efficiently for the lifespan of the organism. Here, we used high-speed live imaging of polarized primary RPE monolayers and complementary biochemical techniques to study mechanisms of cellular clearance in the RPE. Specifically, to investigate how the RPE reduces, reuses and recycles, we studied (i) lysosome trafficking and function; (ii) autophagosome trafficking and autophagic flux; and (iii) polarized secretion and intercellular transfer of exosomes. Analysis of live imaging data showed that degradative organelles (late endosomes, lysosomes and autophagosomes) move bidirectionally with speeds consistent with microtubule-based transport. Presence of bisretinoid metabolites like A2E stabilizes microtubules and decreases long-range organelle movement. Outer segment phagocytosis and inhibition of the mammalian target of Rapamycin (mTOR) upregulate canonical autophagy as measured by LC3 lipidation and clearance of autophagic substrates, whereas A2E inhibits autophagosome biogenesis, trafficking and autophagic flux. Since A2E causes cholesterol storage, increasing cholesterol efflux restored organelle traffic and autophagy in the RPE. Differential centrifugation of apical and basolateral media and immunoblotting showed that RPE monolayers secrete exosomes, sub-micron internal vesicles of late endosomes, in a polarized manner. Exosomes released by A2E-laden cells were enriched in proteins involved in inflammation and immune response. Live imaging showed that RPE monolayers take up exosomes released by other cells, suggesting that these vesicles participate in intercellular communication. Our studies provide a detailed portrait of organelle functions responsible

for reducing, reusing and recycling in the RPE, which are critical for maintaining a healthy climate within the retina.

IRB Status: None

Disclosures:

APARNA LAKKARAJU, PHD: No financial relationships to disclose

O270

THE CONTRIBUTION OF MELANOREGULIN TO MICROTUBULE-ASSOCIATED PROTEIN 1 LIGHT CHAIN 3 (LC3) ASSOCIATED PHAGOCYTOSIS BY THE RETINAL PIGMENT EPITHELIUM

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The degradation of shed photoreceptor outer segments by the retinal pigment epithelium (RPE) is necessary for photoreceptor renewal, function and survival. Phagocytic uptake, phagosome maturation and subsequent degradation of OS content requires a coordinated series of process regulated by several intracellular proteins. One such protein MREG is necessary in the complete digestion of photoreceptor outer segments (POSs); loss of MREG results in incomplete degradation of opsin positive phagosomes as well as lipofuscin accumulation over time. Here we explore the hypothesis that MREG-dependent processing of POS phagosomes links aspects of both autophagic and phagocytic processes in a pathway known as LC3-dependent phagocytosis (LAP). Opsin positive phagosomes associated with LC3 as well as MREG both *in vivo* (by immuno-EM) as well *in vitro* (by confocal imaging). The association of LC3 with OS was independent of nutrient deprivation mediated by rapamycin and dependent on the presence of Atg5. Phagosome maturation studies suggest that MREG is required for LC3 association with POS. Furthermore, the mechanism of MREG action is likely through an interaction with LC3, as determined by co-localization and immuno-precipitation studies. Lastly, the consequences of defective MREG-LC3 association results in the accumulation of cholesterol and 7-ketocholesterol. We posit that POS degradation is a complex process likely requiring convergence of the phagocytic and autophagic pathways in a process that involves LC3 and MREG.

IRB Status: None

Disclosures:

KATHLEEN BOESZE-BATTAGLIA, PHD: No financial relationships to disclose

O271

ROLE OF KINESIN-1 IN THE TRAFFICKING OF PHAGOSOMES AND ENDOSOMES IN THE RPE

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The digestion of phagosomes, derived from the ingestion of outer segment disk membranes, is one of the major tasks to be performed by the RPE. Every day around 10% of the length of the outer segment is shed and digested, and each RPE cell is in contact with multiple photoreceptor cells. The molecular mechanisms and partners involved in ingestion have been fairly well studied, but the molecular motors required for the trafficking of the phagosome and organelles that lead to its complete degradation are mostly unknown. To address these questions we made use of high-speed live cell imaging in primary RPE cultures from wild type and mutant mice. Detailed analyses of our live cell imaging data, combined with other imaging techniques, indicate a role for the microtubule motor, kinesin-1, in the transport of phagosomes. Kinesin-1 was observed to associate with phagosomes. In the absence of functional kinesin-1, phagosome motility and digestion are impaired. Kinesin-1 was also found to associate with late endosomal compartments. We conclude that kinesin-1 is important in the degradation of phagosomes by its involvement in the transport of phagosomes from the apical RPE into the cell body, and potentially in the phagosome-endosome interaction.

IRB Status: None

Disclosures:

MEI JIANG, PHD: No financial relationships to disclose

O272

REMOVAL OF RPE LIPOFUSCIN BY CYCLODEXTRINS

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CORNELL UNIVERSITY

Accumulation of lipofuscin bisretinoids (LBs) in the Retinal Pigment Epithelium (RPE) occurs through phagocytosis of photoreceptor outer segments and is a likely cause of retinal degeneration in genetic blinding diseases (e.g. Stargardt, Best) and a possible etiological agent for age related macular degeneration (AMD). Currently, there are no approved treatments for these diseases; hence, agents that efficiently remove LBs from RPE would be valuable therapeutic candidates. We show that beta cyclodextrins (β -CDs) bind LBs and protect them against light-induced and spontaneous oxidation. Computer modeling and biochemical data support a model in which β -CD encapsulate the retinoid arms of LBs within their hydrophobic cavity. Importantly, β -CD treatment reduced by 73 and 48 % the LB content of RPE cell cultures and of eyecups obtained from Abca4-Rdh8 double knock-out (DKO) mice, respectively. Furthermore, intravitreal administration of β -CDs reduced significantly the content of bisretinoids in the RPE of DKO animals. Thus, our results demonstrate the effectiveness of β -CDs to complex and remove LB deposits from RPE cells. We are currently characterizing the mechanisms involved in CD-mediated lipofuscin removal. Understanding these mechanisms may help

develop novel prophylactic approaches for retinal disorders elicited by LBs.

IRB Status: None

Disclosures:

ENRIQUE RODRIQUEZ-BOULAN, MD: No financial relationships to disclose

O273

PHAGOSOME MATURATION IN RETINAL PIGMENT EPITHELIAL CELLS IN HEALTH AND RETINAL DEGENERATIVE DISEASE

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UNIVERSITY COLLEGE LONDON INSTITUTE OF OPHTHALMOLOGY¹; IMPERIAL COLLEGE, LONDON²; UNIVERSITY COLLEGE LONDON INSTITUTE OF CHILD HEALTH³

A failure to efficiently degrade phagocytosed photoreceptor outer segments by retinal pigment epithelial cells can occur in retinal degenerative disease and aging. We have devised assays that allow the identification of sequential stages of phagosome maturation and allow phagosome: endosome interactions to be monitored. Proteolytic processing of the C-terminus of rhodopsin is co-incident with interaction with endosomes, and occurs before fusion with the lysosome and degradation of outer segment discs. This processing also occurs in macrophages that have phagocytosed rod outer segments and may provide a simple readout of phagosome: endosome interaction that can be used to dissect the molecular mechanisms regulating phagosome maturation in multiple professional phagocytes. Furthermore, these assays have allowed the characterization of defects in phagosome maturation and degradation in mouse models of retinal degenerative disease and the investigation of the relationship between defects in phagosome maturation, intracellular lipofuscin accumulation and extracellular deposit formation.

IRB Status: None

Disclosures:

CLARE FUTTER, PHD: No financial relationships to disclose

O274

ESSENTIAL ROLE OF THE SODIUM/HYDROGEN EXCHANGER 8 IN THE RETINA

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The sodium (Na⁺)/hydrogen (H⁺) exchangers (NHEs) are a family of integral membrane proteins that mediate the electro-neutral exchange of H⁺ with Na⁺ or K⁺. Nine mammalian NHEs (NHE1–NHE9) have been identified; these proteins play broad physiological functions in intracellular pH homeostasis. We have found that NHE8, one of the organellar NHEs that regulate

medial/trans-Golgi pH and intracellular trafficking and control sodium uptake in the kidney and intestine, is essential for the function of the retina. To understand the role of NHE8 in the retina, we have studied its molecular and cellular properties and characterized the retinal phenotype in the NHE8 knockout mice. NHE8 is abundantly expressed in the retinal tissues by RT-PCR; immunostaining reveals the expression of NHE8 proteins in retinal photoreceptor cells, ganglion cells and RPE cells. Both heterozygous and homozygous NHE8 knockout mice are viable with normal appearance. Fundus examination reveals depigmented patches in the retinas of homozygous knockout mice. Histology and flat-mount RPE staining further show disorganized and attenuated RPE layers in the homozygous mutant mice. At 3 weeks of age, although the photoreceptor cell layers remain relatively normal, mutant retinas display drastic abnormalities, including degenerating RPE cells and pigmented round structures in the subretinal space. Histology data also show progressive retinal degeneration as mice age, evidenced by a loss of photoreceptor cells and the shortening of outer and inner segments. These data suggest NHE8 plays essential roles in the function of RPE cells. We are in the process to test our hypothesis that NHE8 controls the pH of Golgi and/or endosomes in RPE cells, and an imbalance of the pH in these intracellular organelles impairs the function of RPE cells thus leading to retinal degeneration.

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IRB Status: None

Disclosures:

CHUN-HONG XIA, PHD: No financial relationships to disclose

RP03 – Very Long Chain Fatty Acids in Ocular Health and Disease

O275

INTER-RELATIONSHIPS OF HUMAN RETINAL VLC-PUFAS WITH BIOMARKERS OF DIETARY LIPID INTAKE

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Very long-chain polyunsaturated fatty acids (VLC-PUFAs) are unusual non-dietary lipids derived from shorter chain dietary polyunsaturated fatty acids. They are uniquely found in retina and few other tissues. Genetic defects in ELOVL4, the key enzyme in VLC-PUFA synthesis are responsible for dominant Stargardt disease (STGD3), and our laboratory has shown that low retinal levels of VLC-PUFAs and abnormally low n-3/n-6 ratios are also found in age-related macular degeneration (AMD) donor eyes relative to age-matched controls, suggestive of a possible role of dietary lipid intake in the pathogenesis of AMD. It is unknown, however, whether dietary intake of VLC-PUFA precursor lipids can beneficially influence VLC-PUFA profiles in retinas from humans without ELOVL4 mutations. We therefore analyzed lipid profiles of 44 normal human donor retinas by gas chromatography mass spectrometry (GC-MS) and compared them to the donors' serum, red blood cell (RBC), and orbital fat lipid profiles which are well established biomarkers

of short-term, medium-term, and long-term lipid consumption, respectively. We found that there was a statistically significant correlation of serum and RBC levels of VLC-PUFA precursors with total retinal VLC-PUFAs ($P < 0.01$) and that n-3/n-6 ratios of all three biomarkers for dietary fat intake were highly predictive of the n-3/n-6 ratios of retinal VLC-PUFAs ($P < 0.001$). The influence of dietary fat intake on retinal lipid profiles was further established in one outlier donor known to have consumed 7 grams of fish oil daily for at least 18 months prior to death. Not only were his biomarkers of polyunsaturated lipid intake and retinal levels of LC-PUFAs and VLC-PUFA much higher than the other donors, but the n-3/n-6 ratio in his retina VLC-PUFAs was also more than twice as high as any other donor.

Support: Macula Vision Research Foundation and Beckman Initiative for Macular Research

IRB Status: Verified

Disclosures:

PAUL BERNSTEIN, MD, PHD: No financial relationships to disclose

O276

VERY LONG CHAIN FATTY ACIDS: MORE THAN NEEDS THE EYE

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Mutations in Elongation of very long chain fatty acids-4 (ELOVL4) cause autosomal dominant Stargardt macular dystrophy (STGD3) as well as neurological and skin disorders. ELOVL4 is an elongase essential for biosynthesis of very long chain fatty acids (VLCFA) found in the retina, brain, skin and testis. However, the mutant-ELOVL4 lacks these biosynthetic capabilities. We hypothesize that expression of mutant-ELOVL4 affects VLCFA biosynthesis which alters photoreceptor structure and function leading to retinal degeneration in STGD3, and that expression of a single copy of mutant-ELOVL4 in the absence of normal ELOVL4 exacerbates the rate of retinal dysfunction. We generated conditional *Elovl4* Knock-In mice (*cKI*) that express only the mutant-ELOVL4, but not wild-type ELOVL4, in photoreceptor cells by crossing *CHX10-Cre* with *floxed-Elovl4* mice to generate *Cre⁺/Elovl4^{flx/flx}*. We then crossed the *Cre⁺/Elovl4^{flx/flx}* mice with *Elovl4* Knock-In (*KI*) mice to generate 4 genotypes: *Cre⁺/Elovl4^{flx/stgd3}* (*cKI*), *Cre⁺/Elovl4^{flx/stgd3}* (*KI*), *Cre⁺/Elovl4^{flx/wt}* (*Het*) and *Cre⁺/Elovl4^{flx/wt}* (*WT*) on which we performed functional, biochemical, and histological analyses at 4 and 6 months, and at 1, 1.5, and 2 years of age. The sum of retinal phosphatidylcholine very long chain polyunsaturated fatty acids (PC-VLC-PUFAs) was significantly reduced in the *cKI* (0.58%) compared to *WT* (7.4%). At 2 years of age, the effect of the mutant ELOVL4 on retinal PC-VLC-PUFA was evident as the *KI* had 2.7% compared to 5.7% in *Het* and 7.4% in *WT*. We observed an age-dependent decline in

rod but not cone function in the *cKI* mice, which had significantly reduced a- and b-wave amplitude responses compared to *WT*. Scotopic b-wave amplitudes were more significantly reduced in the *cKI* compared to *WT* mice. Photoreceptor outer nuclear layer thickness was reduced in the *cKI* compared to *WT* mice. We conclude that *in vivo*, the mutant-ELOVL4 inhibits retinal VLC-PUFA biosynthesis and that depletion of VLCFA with age affects photoreceptor structure and function.

IRB Status: None

Disclosures:

MARTIN-PAUL AGBAGA, PHD: No financial relationships to disclose

O277

EXPRESSION OF ELOVL4 IN THE MEIBOMIAN GLANDS OF MICE EXPLAINS THE PRESENCE OF EXTREMELY LONG CHAIN LIPIDS IN MEIBUM

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Holocrine meibomian glands are located in the tarsal plates in the eyelids of humans and mammals. The glands secrete meibum, a complex mixture of lipids, onto the ocular surface where it forms the outermost part of the multilayered tear film. Compositional changes in meibum were linked to the onset and progress of dry eye disease. To evaluate the lipid composition of meibum of humans and animals (dogs, rabbits, mice, and others), a range of mass spectrometric and chromatographic techniques was developed and implemented.

Meibomian lipids of all tested species were found to be predominantly based on very ($\geq C18$) and extremely ($>C26$) long chain fatty acid (FA) and fatty alcohols (FAI). Wax and cholesterol esters, (O-acyl)-w-hydroxy FA (OAHFA) and their cholesteryl esters (Chl-OAHFA), 1,2-diacyl diols, and other meibomian lipids were shown to be based on saturated and unsaturated FA and FAI with C18–C34 FA, much of which were branched.

A high degree of enrichment of meibum with lipids of extraordinary length suggests a role for the endoplasmic reticulum FA elongation system in their synthesis. To test this hypothesis, we conducted immunohistochemical evaluation of mouse eyelid tissues. In mice, strong expression of ELOVL4, the only elongase capable of elongating FA longer than C26, was observed in differentiating meibocytes as they progressed from initial accumulation of lipid to their final stage prior to rupture to release lipid into the meibomian duct. This observation supports our hypothesis about the *in situ* biosynthesis of most of the meibomian lipids.

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IRB Status: International

Disclosures:

IGOR BUTOVICH, PHD: No financial relationships to disclose

STARGARDT-3 MACULAR DEGENERATION: DEFECTIVE PROCESSING OF PHOTORECEPTOR OUTER SEGMENTS BY RPE CELLS

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ELOVL4 participates in the condensation reaction of very-long-chain fatty acids (VLC-FA and VLC-PUFA). *ELOVL4* is expressed in photoreceptor cells, and VLC-PUFAs are components of photoreceptor outer segment (POS) phospholipids. Mutations in *ELOVL4* cause autosomal-dominant Stargardt-like disease (Stgdt3). However, the pathogenesis associated to the expression of mutant *ELOVL4* remains elusive. We have previously described a transgenic mouse model in which the expression of mutant *ELOVL4*, containing a 5-bp deletion found in Stgdt3 patients, is driven by the photoreceptor-specific IRBP promoter. Here we have investigated cellular effects in the RPE induced by the phagocytosis of POS membrane from mutant *ELOVL4* mice.

The kinetics of wildtype or mutant POS degradation was determined in vitro on primary RPE cells by pulse-chase experiments and immunolabeling with RHO antibodies. POS digestion was also evaluated in vivo. Oxidative stress, inflammation and structural alterations in the RPE were quantitatively analyzed in vivo by immunohistochemical and biochemical methods.

Transgenic *ELOVL4* mice had more POS in the cytoplasm of RPE cells than control mice 2.5 h after light-onset. Primary cultures of RPE cells fed with mutant POS exhibited normal POS binding and ingestion, but the rate of POS degradation was impaired. We also detected increased levels of oxidative stress adducts in the RPE, and a dramatic infiltration of activated microglia in the subretinal space. Our results indicate that POS from mutant *ELOVL4* photoreceptor cells are processed more slowly, a characteristic that is likely to lead to RPE pathology. This defect leads to oxidative stress in the RPE and inflammation in the retina.

IRB Status: Verified

Disclosures:

JULIAN ESTEVE-RUDD, PHD: No financial relationships to disclose

VERY LONG CHAIN SPHINGOLIPIDS AND VASCULAR PERMEABILITY IN DIABETIC RETINOPATHY – THE “SKIN EFFECT” IN THE EYE

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The results of clinical trials revealed strong association between dyslipidemia and development of diabetic retinopathy. However, mechanism(s) of lipid induced retinal pathology are not elucidated. Retina has a unique lipid profile with the highest levels of n3 polyunsaturated fatty acids (PUFA) and very long chain (VLC) PUFA in the body. Additionally, retina is rich in very long chain ceramides. This unique profile is due to very high expression level of fatty acid elongases. Among retinal elongases Elov14 shows the greatest expression level, followed by Elov12, Elov11 and Elov16. Elov14 synthesizes \geq C26 saturated and polyunsaturated fatty acids (PUFA). Diabetes induced dramatic downregulation of *ELOVL4*. Decreased *ELOVL4* expression or function has been associated with multiple retinal disorders, including blood-retinal barrier breakdown, in animal and human studies. Moreover, depletion of ceramides with very long chain fatty acids (\pm C28) in the epidermis of Elov14^{-/-} mice led to the breakdown of water permeability barrier function of skin. We hypothesized that very long chain ceramide production by *ELOVL4* is essential for blood-retinal barrier function and downregulation of Elov14 in diabetes contributes to increased retinal vascular permeability and degeneration. *ELOVL4* overexpression in human retinal pigment epithelial (RPE) and human retinal endothelial cell (HREC) culture models of the blood-retinal barrier, we found increased levels of C26 fatty acids incorporated into ceramide while decreasing shorter chain ceramides in these cells. Altered sphingolipid content by *ELOVL4* decreased RPE cell activation in response to stimulation by the pro-inflammatory cytokine IL-1 β . Intravitreal delivery of *ELOVL4*-AAV2 to retinas of STZ diabetic rats increased retina very long chain ceramides, reduced retinal vascular activation, blunted diabetes-induced retinal vascular permeability, and increased endothelial expression of blood-retinal barrier components. Taken together these data indicate that *ELOVL4* overexpression prevents increase in vascular permeability and vascular degeneration in diabetic retina through modulation of sphingolipid metabolism.

IRB Status: Verified

Disclosures:

JULIA V. BUSIK, PHD: No financial relationships to disclose

CREL IS AN INTRACELLULAR MESSENGER OF THE ESSENTIAL DOCOSAHEXAENOIC ACID-DERIVED MEDIATOR NEUROPROTECTIN D1 IN RPE CELL SURVIVAL

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LOUISIANA STATE UNIVERSITY HEALTH SCIENCES CENTER

Neuroprotectin D1 (NPD1) induces cell survival in uncompensated oxidative stress conditions. Therefore it is critical for photoreceptor cell integrity. The molecular principles underlying this protection remain unclear. We found that, in ARPE-19 cells and in human primary cultures of retinal pigment epithelial (RPE) cells, NPD1 triggers nuclear translocation and cREL expression that, in turn, mediates BIRC3 synthesis. NPD1 induces NF- κ B by an alternate route to canonical signaling, and the opposing effects of TNFR1 and NPD1 on BIRC3 expression likely are not due to interaction/s between NF- κ B pathways. Our data suggest that

cREL regulates a cluster of NPD1-dependent genes after cREL nuclear translocation. BIRC3 silencing prevents NPD1 induction of survival against oxidative stress, suggesting non-redundancy. Moreover, brain NPD1 biosynthesis and selective neuronal BIRC3 abundance are increased by DHA after experimental ischemic stroke followed by remarkable neurological recovery. Thus, NPD1 bioactivity is a decision-making event that counter-regulates key gene transcription whereby cRel expression and nuclear translocation regulate RPE cell integrity when confronted with potential disruptions of function, such as in retinal degenerations.

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IRB Status: Approved

Disclosures:

NICOLAS BAZAN, MD, PHD: No financial relationships to disclose

RP04 – RPE and the Complement System

O281

INTERACTIONS OF COMPLEMENT FACTOR-H AND FACTOR H LIKE PROTEIN-1 WITH MACULAR TISSUE

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Genetic and eye tissue analyses implicate dysregulation of the alternative complement pathway at the RPE/Bruch's membrane interface as being important in AMD pathogenesis. Genetic alterations in and around the complement factor H (CFH) gene are a major AMD risk factor, with one important polymorphism resulting in a Y402H substitution in CFH. CFH, inhibits complement activation on cell surfaces and the extracellular matrix. Factor H like protein-1 (FHL-1) is a shortened splice variant which retains all of the regulatory activity of full-length CFH, and FHL-1 is subject to the Y402H polymorphism. Using specific antibodies against CFH and FHL-1 we investigated their localization in human macular tissue. We found strong labeling for FHL-1 throughout the thickness of Bruch's membrane and weaker labeling in the choroidal stroma. CFH labeling was confined to the surfaces of Bruch's membrane and was prominent in the choroidal stroma. Drusen showed surface labeling with CFH, but within druse labeling was confined to FHL-1. An investigation into RPE expression by qPCR demonstrated 20x more FHL-1 expression than CFH expression. Diffusion experiments demonstrated that FHL-1 could pass through Bruch's membrane, whereas CFH could not. We have previously shown that the Y402H polymorphism affects the binding of CFH to macular tissue, and this is largely due to the 402H form binding relatively poorly to heparan sulfate in Bruch's membrane. We found the same pattern for FHL-1 binding. Furthermore, we have demonstrated decreased levels of heparan sulfate in Bruch's membrane with ageing. We conclude that as well as full-length CFH, FHL-1 is important in regulating complement activation

in Bruch's membrane and that the age-related loss of heparan sulfate in Bruch's membrane will result in decreased FHL-1 and CFH binding, and may then predispose to AMD.

IRB Status: International

Disclosures:

PAUL BISHOP, FRCOPHTH, PHD: No financial relationships to disclose

O282

TARGETED DELETION OF COMPLEMENT FACTOR H IN THE MOUSE RPE

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There is compelling genetic and biochemical evidence that dysregulation of the complement system contributes to the cellular pathogenesis of age-related macular degeneration (AMD). Complement factor H (CFH) is a major player in this context, with the Y402H variant of CFH being linked to increased risk of disease. Previously we reported the retinal phenotype of aged mice lacking CFH and observed mild phenotypic changes in the retina, some of which were similar to those observed in AMD. One limitation of the previous study is that the global CFH knockout mouse is almost completely lacking in complement C3, making it impossible to evaluate any specific role that CFH might play in regulating the activity of C3 and other complement factors in the retina. To address this problem, and to gain insight into the biological significance of local production of CFH in the retina, we have now generated mice containing a targeted deletion of CFH in the retinal pigment epithelium. These mice contain normal circulating levels of both CFH and C3, primarily due to synthesis of both in the liver, yet lack the local production of CFH in the retina, enabling us to investigate the consequences of this on the deposition of C3 and its breakdown products in the retina and Bruch's membrane. This mouse model may provide us with a better understanding of the biological role of CFH in the retina and may reveal why, given that complement variants have a systemic reach, only the retina is affected in disease.

IRB Status: None

Disclosures:

STEPHEN MOSS, PHD: No financial relationships to disclose

O283

LESSONS LEARNED FROM MURINE MODELS OF COMPLEMENT DYSREGULATION

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Age related macular degeneration (AMD) is the leading cause of blindness in the elderly in Western societies. Genetic

analyses have established a strong connection between AMD and components of the alternative complement system, in particular the regulator complement factor H (CFH), where a tyrosine (Y) to histidine (H) exchange in domain 7 dramatically increases the risk for AMD. To study the function of CFH in a relevant model system, we generated bacterial artificial chromosome transgenic mouse lines expressing the full-length protein coding for either the normal (Y402) or the AMD-risk associated (H402) human *CFH* variants, which we then crossed to *cfh* null (*Cfh*^{-/-}) mice. In these animals the human CFH protein functions like its rodent counterpart in the alternative pathway in an expression level-dependent manner. Old *Cfh*^{-/-} mice have an AMD-like ocular phenotype plus kidney defects resembling human dense deposit disease. One of the mouse lines, *CFH*^{Y402}, *Cfh*^{+/+}, expressing the normal human Y402 CFH variant, demonstrated functional and structural protection of the retina and renal phenotype due to the *Cfh* deletion. Impaired visual function, detected as a deficit in the scotopic electroretinographic response, is statistically significantly improved in this transgenic mouse line compared to *Cfh*^{-/-} mice. In addition, the outer nuclear layer (ONL) thickness of the Y402 mice is comparable to age-matched normal controls and is statistically significantly thicker than in *Cfh*^{-/-} mice. Furthermore, there is statistically significantly less sub-RPE deposit accumulation than in the *cfh* knockout based on ultrastructural quantification of basal deposits. The mice provide a validated model system that will be used to elucidate how changes in CFH impact AMD pathogenesis and to test the additive effect of other AMD risk factors.

IRB Status: None

Disclosures:

CATHERINE BOWES RICKMAN, PHD: No financial relationships to disclose

O284

COMPLEMENT REGULATION AND DYSREGULATION AS A FUNCTION OF GENOTYPE IN CULTURED HUMAN RETINAL PIGMENT EPITHELIUM

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It is widely agreed that the sequence variants, Y402H and I62V in the gene for complement factor H (CFH) are strongly associated with Age Related Macular Degeneration (AMD). However, the cell pathobiology that ensues from the consequent change in protein amino acid sequence has remained elusive. CFH, a negative regulator of complement, is secreted not only by the liver but by the retinal pigment epithelium (RPE) and may therefore be essential for local control of complement activity at the level of the RPE/choriocapillaris. ABCA4, is a phospholipid translocator in photoreceptor rod and cone outer-segments (OS) that clears retinaldehyde following a photobleach and prevents the accumulation of toxic *bis*-retinoids in photoreceptors which, if not removed, can result in their buildup in the RPE as a consequence of OS phagocytosis. Employing genotyped, cultured human RPE, we documented the correlation between *CFH* haplotype and *bis*-retinoid-based,

inappropriate complement activation and attack on RPE cells. RPE carrying the AMD-predisposing *CFH* haplotype, (HH402/VV62) permitted robust complement attack on RPE following challenge with *bis*-retinoid-containing *Abca4*^{-/-} OS harvested from a mouse model of recessive Stargardt disease. This complement activation required factor B, implicating the alternative pathway. Inappropriate attack was not observed on RPE carrying the AMD-protective *CFH* haplotype (YY402/II62) following exposure to *Abca4*^{-/-} OS. Complement attack on RPE was observed only when challenged with *Abca4*^{-/-} OS, not *Abca4*^{+/+} OS. Our results implicate *bis*-retinoid accumulation in hRPE and subsequent release of more water soluble, oxidized downstream byproducts as a potential trigger for inappropriate complement attack of RPE that is genetically predisposed toward the development of AMD, whereas cells with a CFH protective genotype are capable of fending off complement attack.

IRB Status: Verified

Disclosures:

DEAN BOK, PHD: No financial relationships to disclose

O285

HOMOZYGOUS RISK FOR ARMS2 AND CFH CORRELATES WITH INCREASED MTDNA DAMAGE: DEVELOPING PERSONALIZED MEDICINE TO TREAT AGE-RELATED MACULAR DEGENERATION

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UNIVERSITY OF MINNESOTA¹; UNIVERSITY OF WASHINGTON²; UNIVERSITY OF CALIFORNIA IRVINE³

Association of high risk age-related macular degeneration (AMD) genes with diverse biological pathways suggests different mechanisms lead to AMD. Therefore, a single therapy may not be universally effective. Recent evidence suggests defects in mitochondria (mt) of the retinal pigment epithelium (RPE) may be a key pathogenic event in some AMD patients. The purpose of this study is to determine if individuals with a specific genetic background have a greater propensity for mtDNA damage. This information could provide the basis for developing a pharmacogenetics approach to treat AMD using mt-targeted drugs. Donor eyes were obtained by the Minnesota Lions Eye Bank following consent of the donor or family for medical research. Fundus images were used to evaluate the stage of AMD using the Minnesota Grading System. Genomic DNA was isolated from RPE harvested from the macula region. RPE mtDNA damage was measured using a long extension polymerase chain reaction in 76 donors with AMD and 42 age-matched controls. Genotype analyses were performed for the major AMD risk alleles CFH, ARMS2, APOE, C3, LIPC, and VEGF. Donors with homozygous risk alleles for ARMS2 and CFH had significantly more mtDNA damage compared with donors having a heterozygous or wild type genetic profile. Consistent with epidemiological studies, the majority of donors (~85%) harboring the homozygous risk genes exhibited an AMD disease phenotype. No association was found for SNP high risk

variants of APOE, VEGF, LIPC, or C3. This study provides the rationale for a more personalized approach for treating AMD by revealing significant correlates between the CFH and ARMS2 risk genes and accelerated mitochondrial damage. Patients with these homozygous risk alleles may benefit from therapies targeted at stabilizing the mitochondria and reducing free radical generation in the RPE.

IRB Status: Verified

Disclosures:

DEBORAH FERRINGTON, PHD: Contracted Research relationship with Stealth Peptides

O286

A CELL CULTURE MODEL FOR GEOGRAPHIC ATROPHY

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UC SANTA BARBARA, NEUROSCIENCE RESEARCH INSTITUTE

Geographic atrophy (GA) is the end stage of dry age-related macular degeneration (AMD) where central vision is lost. It is a slowly progressing, irreversible disease and there is currently no cure or clear explanation of its cause, although age, cigarette smoking (oxidative stress), and genetics (e.g. TRL3) are implicated. Since there are very few *in-vitro* models to study macular degenerations, we introduce a cell-culture system that models the progression of geographic atrophy.

GA is different from wet AMD in which choroidal neo-vascularization (CNV) is the end point. Although both CNV and GA begin as dry AMD, it is unclear why some people advance to one form of AMD or the other. The GA stage of AMD is characterized by circular patches of partial or complete RPE de-pigmentation leading to exposure of the choroid under the RPE. In our model, retinal pigmented epithelium (hRPE) cells are grown on a porous support matrix (Millipore HA cell culture inserts). Then, using AnnexinV / PI labeling and fluorescence microscopy, we show how stress-induced RPE apoptosis creates a characteristic pattern of spreading cell death with similarities to GA.

The circular wave of infectious apoptosis leaves an exposed debris-field of extra-cellular detritus in its wake. As the cells degenerate, these sub-cellular deposits are targeted by the complement system, as evidenced by C5b-9 deposition. In addition, we use immunofluorescence confocal microscopy to observe the nuclear localization of known drusen proteins (beta-amyloid, ApoE, and clusterin) in RPE cells after oxidative stress. We show that several drusen-related proteins are delivered to the extra-cellular environment by degraded nuclei. We also demonstrate that cells grown on apoptotic debris never attain a normal morphology. These results illustrate one possible mechanism for progressive macular degeneration and have potential implications for cellular replacement therapies.

IRB Status: None

Disclosures:

DAVID FOREST, MS: No financial relationships to disclose

RP05 – Choroid in Health and AMD

O287

INSULT BY COMPLEMENT: CHOROIDAL ENDOTHELIAL RESPONSE TO MAC DEPOSITION

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Age-related macular degeneration (AMD) is associated with variants in a number of complement cascade genes. A growing body of histochemical, proteomic, and transcriptomic evidence indicates that the membrane attack complex (MAC) of complement is deposited in the aging choriocapillaris and that choriocapillaris endothelial cells are affected very early in the pathogenesis of AMD. We hypothesized that increased activation of the complement cascade in the choroidal endothelium disrupts homeostatic gene expression, pathogenically altering endothelial physiology. To test this hypothesis, we challenged primate choroidal endothelial cells with MAC and assessed changes in gene expression by RNA-Seq. Rhesus macaque choroidal endothelial cells (RF/6A) were treated with normal human serum or heat inactivated serum. Activation of MAC was confirmed using immunocytochemistry. RNA was extracted and subjected to RNA sequencing. Alignment to the Ensembl macaque genome assembly (build MMUL_1) and differential expression analysis were performed using the Tuxedo software suite. Gene set analyses were performed using the DAVID website. Validation of a subset of messages was performed using qPCR. Anti-MAC immunocytochemistry identified marked increased of MAC puncta on cells treated with normal serum but not heat-inactivated serum. Scanning electron microscopy revealed numerous blebs on MAC injured cells, consistent with previous reports of MAC clearance by membrane blebbing. RNA-Seq revealed a strong response in gene expression, with active MAC increasing expression of 214 transcripts while decreasing expression of 139 (q-value ≤ 0.001 , minimum three-fold difference). Differentially expressed genes were enriched for terms associated with extracellular secretion, growth factors, cytokine activity, extracellular matrix, inflammatory response, and angiogenesis. Complement activation disrupts the molecular homeostasis of primate choroidal endothelial cells, and alters gene expression in patterns consistent with the pathogenesis of AMD, suggesting avenues for further investigation of early AMD.

IRB Status: Verified

Disclosures:

S. SCOTT WHITMORE, MS: No financial relationships to disclose

O288

PERIVASCULAR MURAL CELLS AND DENDRITIFORM MYELOID CELLS IN THE MOUSE CHOROID – MORPHOLOGY, DISTRIBUTION, AND INTERACTIONS

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The choroid of the eye consists of a specialized vascular bed providing vascular supply to the retina and playing roles in thermoregulation, eye growth, and emmetropization. Significantly, choroidal changes feature prominently in retinal diseases, particularly AMD. However, the description of perivascular components of the choroid and how they contribute to choroidal function and pathology are incompletely understood. We have employed transgenic mouse models in which GFP is specifically expressed in perivascular mural cells (α -smooth muscle actin (SMA) promoter driving GFP) or choroidal myeloid cells (CX3CR1 promoter driving GFP) to examine the distribution, morphology, behavior of these cell types. We found that perivascular mural cells demonstrate marked morphological diversity, ranging from densely-packed circumferentially-oriented cells that provide complete vascular coverage in primary arteries to widely-spaced stellate-shaped cells that are distributed sparsely over terminal arterioles. Immunopositive for contractile proteins α -SMA and desmin, perivascular mural cells demonstrate calcium-regulated contractile movements that can subserve varying requirements for vasoregulation in the choroid. On the other hand, perivascular myeloid cells, comprising most of the resident immune cell population in the choroid, were widely distributed across the choroid and demonstrated close associations with choroidal vessels that varied with their position in the vascular tree. Notably, myeloid cells associated with choroidal arteries and arterioles appeared as elongated cells flanking the long axes of vessels, whereas those associated with the choriocapillaris were distributed as a layer of stellate cells on the scleral but not vitreal choriocapillaris surface. While stationary in position, dendritiform myeloid cells demonstrated the rapid process dynamism well suited to comprehensive immunosurveillance of the perivascular space. Taken together, perivascular mural and myeloid cells have highly-structured morphological and behavioral features that likely underlie functional contributions to the choroid. Work is ongoing investigating the interactions between these two cell types and their contributions to function and pathology in the choroid.

IRB Status: Approved

Disclosures:

WAI WONG, MD PHD: No financial relationships to disclose

O289

CD68+ AND CD163+ MACROPHAGES IN THE NORMAL HUMAN ADULT CHOROID

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A preliminary survey was undertaken to describe the distribution of CD68+ and CD163+ macrophages in the normal adult human choroid. CD68 (monoclonal mouse anti-human, clone 514H12) and CD163 (monoclonal mouse anti-human, clone 10D6) immunohistochemistry was performed on formalin fixed, paraffin embedded tissue from 6 human eyes (4 donors, 52

to 59 years) with no history of ocular disease and no gross or microscopic pathology, using the Leica Bond II automated protocol and Leica Bond Polymer Refine Red Detection kit. Cell counts (average per 5 high power fields - HPF - for each region in each eye) and photography was performed using an Olympus BX51 brightfield microscope and DP26 camera. Mean cell counts were compared and differences calculated with 95% confidence intervals. CD163+ macrophages outnumbered CD68+ macrophages in both central choroid (mean difference 6.67 ± 3.20 cells/5HPF; 95% CI 3.31 – 10.03) and peripheral choroid (mean difference 6.02 ± 1.39 cells/5HPF; 95% CI 4.56 – 7.47). Within central and mid peripheral choroid, Bruch's membrane (BrM) was devoid of macrophages. Cell counts for both CD68+ and CD163+ cells increased with increasing distance away from BrM, with the majority of choroidal macrophages found in Sattler's and Haller's layers. However, CD163+ macrophages were seen abutting and apparently traversing BrM in the outer periphery, particularly at the region of the ora serrata. CD163+ macrophages, but not CD68+ macrophages, extended their processes into small hard drusen, found in the mid periphery of two eyes. Elsewhere in the uveal tract, the ciliary body stroma recorded the highest CD163+ and CD68+ macrophage counts. These observations suggest the human choroid harbours different macrophage subpopulations, which appear to be excluded from Bruch's membrane except in the region of the ora serrata or in the vicinity of small hard drusen.

IRB Status: Approved

Disclosures:

SVETLANA CHEREPANOFF, MBBS PHD: No financial relationships to disclose

O290

INFLAMMATORY CELLS OF CHOROID IN AGING AND AMD

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The human choroid is severely affected by age-related macular degeneration (AMD). There is convincing evidence that the choroid thins in AMD (Spaide et al, Am.J.Ophthalmol., 2009), suggesting that the cells of choroid die. One cell type lost in AMD choroid is the choriocapillaris endothelial cell. The choriocapillaris (CC) atrophies in both wet and dry AMD (McLeod et al, IOVS, 2009). In an effort to understand this death of CC, we evaluated the inflammatory cells of choroid. Nonspecific esterase enzyme histochemistry (Lutty et al, A.J.Pathol. 1997) was used to label granulocytes, which include polymorphonuclear leukocytes (PMNs) and mast cells, in human aged and AMD choroids. Alkaline phosphatase enzyme histochemistry was used to assess endothelial cell viability and determine the density of the vasculature (McLeod et al, IOVS, 1994). PMNs were almost exclusively intraluminal in all subjects and aged controls had comparable numbers to AMD subjects. Mast cell numbers were comparable in aged controls and AMD subjects in non-submacular areas. However, there were increased mast cells in submacular choroid of GA subjects compared to

controls, many of which were degranulated. Degranulation releases proteases, vasogenic factors, and cytokines, which may contribute to the atrophy of choroid in this area. Preliminary studies on fellow choroids using the macrophage/microglia marker Iba-1 and UEA lectin for viable endothelium demonstrated the prominence of these cells in choroid. Normally all Iba-1⁺ cells are posterior to choriocapillaris. Those in Sattler's layer are ramified while ones in Haller's layer are round or fusiform. In our preliminary study of these preparations, there was no increase in Iba-1⁺ cells in AMD choroid, except in areas with CNV. In conclusion, there is an increase in the number of mast cells, degranulated and nondegranulated, in AMD submacular choroid. Their degranulation and release of chemical mediators may contribute to cell death and thinning of choroid.

IRB Status: Verified

Disclosures:

GERARD LUTTY, PHD: No financial relationships to disclose

O291

THE ROLE OF PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR (PPAR β/δ) IN CHOROIDAL ANGIOGENESIS

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Age related macular degeneration (AMD) is a leading cause of blindness in the developed world. The exudative form of AMD is characterized by abnormal growth of the choroidal vasculature, leading to leaky vessels that proliferate into the sub-retinal space. This choroidal neovascularization is associated with increased levels of vascular endothelial growth factor (VEGF) in ocular tissues, possibly secreted from retinal pigmented epithelial cells (RPE). Anti-VEGF therapy is currently used to treat AMD, but it has demonstrated limited efficacy in some patient populations. The transcription factor peroxisome proliferator activated receptor (PPAR β/δ) may provide a better target for therapy. PPAR β/δ is known to regulate energy homeostasis, lipid catabolism, and glucose metabolism, but evidence also suggests that it plays a role in retinal angiogenesis. To determine the role of PPAR β/δ in choroidal angiogenesis, primary murine RPE and ARPE-19 cells were exposed to hypoxia and treated with increasing concentrations of the PPAR β/δ antagonist GSK0660. Secreted VEGF was reduced, dose-dependently, by GSK0660 in both cell types ($p < 0.025$). Proliferation and tube formation of primary human choroidal endothelial cells (CEC) were assessed in the presence of increasing concentrations of GSK0660 while vascular sprouting in the presence or absence of GSK0660 was analyzed in mouse choroid explants. There were dose-dependent reductions in the amount of proliferation ($p = 0.0001$) and the extent of tube formation of CEC ($p = 0.0007$) in the presence of GSK0660. Finally, GSK0660 inhibited choroidal vascular sprouting by 30% ($p = 0.025$). Our findings indicate that inhibition of PPAR β/δ may reduce choroidal angiogenesis both by inhibiting secretion of VEGF by RPE as well as inhibiting angiogenic behaviors of CEC. Since PPAR β/δ appears to play a role in multiple aspects of the

neovascular response, it may have advantages over anti-VEGF or other single target therapies against AMD.

IRB Status: None

Disclosures:

SARA SAVAGE, BS: No financial relationships to disclose

RP06 – Signaling Pathways and Organelle Dynamics

O292

REGULATION OF MERTK ACTIVITY DURING RPE PHAGOCYTOSIS BY EXTRACELLULAR PROTEINS

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INSTITUT DE LA VISION

Regulation of shed photoreceptor outer segment extremities (POS) by retinal pigment epithelial (RPE) cells is a diurnal process that is highly regulated at the molecular level. *In vivo*, internalization of POS via the MerTK receptor is triggered through intracellular pathways initiated by the couple MFG-E8 ligand – α 5 β 1 integrin receptor. However, in their absence the rhythm of phagocytosis is lost but internalization still occurs. Recent data showed that both MerTK cognate ligands, Gas6 and Protein S, are required together but their exact individual contribution remains unclear. Our current data suggest that only Protein S is expressed rhythmically in the retina, while Gas6 expression is stable with time. In addition, MerTK activity appears to be downregulated through cleavage of its extracellular domain. Thus, we investigated both MerTK ligands and putative proteases during POS phagocytosis by RPE cells. Effects of various concentrations of ligands used either alone or in combination during *in vitro* phagocytosis assays are more marked on POS internalization than binding. While Protein S increases POS phagocytosis in a dose-dependant fashion, augmenting Gas6 doses blocks phagocytosis. When used together, their effects are counterbalanced. In contrast, in J774 macrophages, which share the same molecular machinery, all ligands have stimulatory effects that are additive when combined. These results suggest a tissue-specific role for MerTK ligands in the retina. Among the protease candidates tested, HtrA1 and members of the ADAM family of metalloproteases, only ADAM17 appears to be able to cleave MerTK *in vitro*, albeit to a limited extent. Strikingly, addition of Gas6 seems to increase MerTK cleavage while Protein S inhibits it. Moreover, ADAM17 is the only protease to be expressed in RPE microvilli on retinal sections. Taken together, our results suggest that 2 extracellular pathways contribute to downregulate MerTK activity during POS phagocytosis, possibly limiting its time frame of action.

IRB Status: None

Disclosures:

EMELINE NANDROT, PHD: No financial relationships to disclose

SIGNALING DOWNSTREAM OF MERTK IN RPE PHAGOCYTOSIS

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Phagocytic uptake of photoreceptor outer segments (POS) by the retinal pigment epithelium (RPE) requires activation of the receptor tyrosine kinase MERTK that propagates downstream signaling through the activation of SH2-domain proteins. Our studies have shown that MERTK recruits multiple SH2-domain proteins in the RPE, including Src family kinases (SFKs) and p85-alpha, the regulatory subunit of type I phosphatidylinositol 3-kinase (PI3K). MERTK activation of SFKs results in tyrosine phosphorylation of the Rab effector GDP dissociation inhibitor alpha (GDI1) during peak phagocytic uptake *in vivo*. GDI1 colocalizes with MERTK and Src in the apical RPE, and on phagosomes in cultured RPE cells resulting from POS feeding. Phosphorylation of GDI1 results in decreased interaction with Rab5 GTPase, a small G protein that participates in membrane trafficking by partitioning between donor and acceptor membranes. The p85-alpha subunit of type I PI3K colocalizes on phagosomes with early endosome antigen 1 (EEA1), a Rab5 GTPase effector that binds to the phosphatidylinositol 3-phosphate lipid anchor and facilitates protein docking and membrane fusion. MERTK-dependent phagocytic uptake activates cellular autophagy as measured by increased conversion of the cytosolic form of LC3 (LC3-I) to the phosphatidylethanolamine conjugate (LC3-II) that is recruited to autophagosomal membranes, which is not seen in the presence of inhibitors that block signaling at the level of the receptor. Our studies thus identify multiple avenues by which MERTK signaling can impact intracellular membrane trafficking essential for the phagocytic mechanism, as well as for RPE homeostasis.

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IRB Status: None

Disclosures:

DEBRA THOMPSON, PHD: No financial relationships to disclose

REGULATION OF AUTOPHAGY AND PHAGOCYTOSIS BY *CRYBA1* IN RETINAL PIGMENT EPITHELIAL CELLS

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The Retinal Pigmented Epithelium (RPE) serves many physiological roles that are crucial for maintaining homeostasis of the retina. RPE cells are among the most active phagocytic cell types in the body, phagocytosing 10% of total photoreceptor volume daily. Moreover, autophagy, a process by which cellular constituents are degraded and recycled as part of normal cellular remodeling, is likely to be of particular importance in post-mitotic cells with high metabolic demand, such as the RPE. Autophagy and phagocytosis are interconnected; as the latter stages of both these pathways require fusion with lysosomes. Incomplete lysosomal degradation and concomitant accumulation of undegraded intracellular material is thought to contribute to lipofuscin formation and RPE cell dysfunction in age-related macular degeneration (AMD). We have recently generated a transgenic mouse model with abnormal lysosomal clearance leading to impaired phagocytosis and autophagy in the RPE. These mice show scattered drusen-like lesions by 4 months of age, hyper-vacuolation due to accumulation of undigested photoreceptor outer segments, and loss of retinal function. Impaired autophagy is also accompanied by hyperactivation of mechanistic target of Rapamycin complex 1 (mTORC1) in these mice. In some cell types, mTORC1 is known to negatively regulate autophagy, and influence lysosomal function by inhibiting the lysosomal transcription factor, TFEB. However mTORC1 signaling mechanisms in the RPE are not understood. We hypothesize that mTORC1 plays an important role in the regulation of lysosomal clearance functions in the RPE and that modulating mTORC1 can have profound implications in inhibiting RPE degeneration. Our preliminary results suggest that overexpression of TFEB in the WT RPE results in enhanced activity of lysosomal proteases like Cathepsin D. These results demonstrate the importance of mTORC1 pathway in lysosomal regulation in RPE. Further studies are underway to understand the involvement of mTORC1 pathway in atrophic loss of RPE during the pathogenesis of AMD.

IRB Status: None

Disclosures:

MALLIKA VALAPALA, PHD: No financial relationships to disclose

ER STRESS SIGNALING IN REGULATION OF THE RPE BARRIER FUNCTION

JACEY MA, Sarah Zhang

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Dysfunction of the blood-retinal barrier (BRB) at the retinal pigment epithelium (RPE) plays an important role in the pathophysiology of diabetic retinopathy and age-related macular degeneration. Reduced expression and/or dislocation of tight junction proteins are the primary cause of BRB disruption and functional defects. However, how the RPE tight junction complex is regulated remains obscure. Recently, we have shown that signaling pathways of the unfolded protein response (UPR) activated by ER stress are important for RPE cell survival. Herein, we investigated the role of ER stress signaling in regulation of RPE tight junctions. The UPR was activated by pharmacological

ER stress inducers thapsigargin and tunicamycin in differentiated ARPE-19 cells. Sublethal dose of thapsigargin reduced ZO-1 expression and impaired tight junction formation. Interestingly, tunicamycin did not show any detrimental effect on tight junctions in normal RPE cells, but caused a remarkable damage in the cells pre-treated with a specific inhibitor of XBP1 activation. Likewise, XBP1 inhibition rendered primary primate RPE cells sensitive to tunicamycin- and thapsigargin-induced tight junction damage. These results suggest that XBP1 activation may be an endogenous protective mechanism in ER stress-related RPE barrier damage. In support of this notion, overexpressing active XBP1 largely reversed the tight junction damage caused by XBP1 inhibition. Furthermore, defects in RPE tight junctions were observed in mouse RPE with down-regulated XBP1 expression. This was accompanied by redistribution of F-actin and upregulation of RhoA expression, which implies a role of Rho/Rho kinase pathway in RPE barrier dysfunction caused by XBP1-deficiency. Taken together, our results suggest that activation of XBP1 by ER stress may play a protective role in the RPE barrier function. Future studies will decipher the mechanisms by which XBP1 regulates tight junction proteins and explore the implications of other UPR pathways in ER stress-associated RPE barrier damage.

IRB Status: None

Disclosures:

JACEY MA, MD: No financial relationships to disclose

O296

REGULATION OF RPE PHENOTYPE BY ANNEXIN A8 AND WNT SIGNALING

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Fenretinide (FR), a retinoic acid derivative, is capable of trans-differentiating retinal pigment epithelial (RPE) cells towards a neuron-like phenotype in culture. Microarray analysis post-FR treatment revealed down-regulation of Annexin (Anx) A8 as well as proteins involved in Wnt signaling in trans-differentiated cells. AnxA8, a calcium-dependent phospholipid-binding protein, is expressed in the RPE cell cytosol, where it is believed to be involved in membrane and cytoskeletal organisation and cell proliferation. The aim here was to analyse the role of AnxA8 and its interaction with Wnt signaling in RPE cell trans-differentiation. Therefore, human RPE cells were seeded at a concentration of 2,200/ml and treated with 3% charcoal dextran-treated foetal bovine serum (FBS) for 24h. 3 μ M FR or vehicle (0.1% dimethylsulfoxide) was added every day for 7 days. As a second approach, AnxA8 was suppressed in RPE cells using short interfering RNA (siRNA). FR and AnxA8 siRNA treatment both induced a decrease in AnxA8 expression and inhibited cell proliferation. It further led to trans-differentiation of ARPE-19 cells into neuron-like cells and a concomitant up-regulation of the neuronal markers Calbindin and Calretinin analysed by qPCR and immunofluorescence. Additionally, expression of Wnt signaling proteins such as β -Catenin, Frizzled-1, Frizzled-4, Wnt2b and Wnt3a was decreased. The effect of FR was partially reversible by activation of Wnt signaling using recombinant Wnt3a or SB216763, a glycogen synthase kinase-3 β inhibitor. Wnt inhibitors, such as Dickkopf-1 and DAPT were not able to reverse the FR effect. These data imply an important role for AnxA8 in maintaining RPE phenotype. Down-regulation of AnxA8 appears to be sufficient for neuronal trans-differentiation of RPE cells and the expression of neuronal markers. Further, the interdependence of AnxA8 and Wnt proteins suggests that AnxA8 might be an important regulator in Wnt signaling.

IRB Status: None

Disclosures:

KATHARINA LUECK, PHD: No financial relationships to disclose

O297

REGULATION OF LYSOSOMAL FUNCTION IN RPE CELLS

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Daily phagocytosis of spent photoreceptor outer segment fragments (POS) by the retinal pigment epithelium (RPE) is a process essential for vision. The phagocytosis process includes three distinct yet coupled phases, recognition and binding, engulfment, and phagolysosomal digestion. Here, we investigated if the membrane scaffolding protein caveolin-1 plays a role in RPE phagocytosis. First, we compared POS phagocytosis by the RPE *in situ* in wild-type mice and mice lacking caveolin-1 specifically in the RPE. We found that lack of caveolin-1 has no effect on the early morning burst of POS uptake but significantly delays phagolysosomal digestion of POS proteins. Studies of RPE cells in culture confirmed a role for caveolin-1 in POS protein digestion and specified that decreasing caveolin-1 impairs lysosomal enzyme activity and increases lysosomal pH. Conversely, overexpressing wild-type caveolin-1 was sufficient to accelerate POS phagolysosomal digestion. Overexpressing a scaffolding mutant form of caveolin-1 that does not reach the plasma membrane was equally effective in promoting POS digestion suggesting that caveolin-1 acts at intracellular sites. Moreover, we found that caveolin-1 co-localizes with acidified POS-opsin phagolysosomes. Taken together, our results suggest a novel role for caveolin-1 in phagolysosomal digestion of POS by RPE cells.

IRB Status: None

Disclosures:

SILVIA FINNEMANN, PHD: No financial relationships to disclose

RP07 – NLRP3 Inflammasome

O298

IL-18 PROCESSING AND AGE-RELATED MACULAR DEGENERATION: A TALE OF TWO STATES

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Age-related macular degeneration (AMD) is the most common form of central retinal blindness in the developed world. Inflammation is known to play a key role in the pathogenesis of AMD. The activation of the NLRP3-inflammasome, has come to the fore as a key player in the development of both "dry" and neovascular ("wet") forms of the disease. The inflammasome regulates the maturation of the pro-inflammatory cytokines IL-1 β and IL-18. We show that IL-18 can regulate choroidal neovascularization (CNV) formation in mice. We observed that exogenous administration of mature recombinant IL-18 has no effect on retinal pigment epithelial (RPE) cell viability, but that over-expression of pro-IL-18 or pro-IL-1 β in RPE cells can cause RPE cell swelling and subsequent atrophy, a process that can be inhibited by the promotion of autophagy. A direct comparison of local and systemic administration of mature recombinant IL-18 with current anti-VEGF-based therapeutic strategies shows that IL-18 treatment works effectively alone and more effectively in combination with anti-VEGF therapy. Recombinant IL-18 therefore represents a novel therapeutic strategy for the treatment of wet AMD.

IRB Status: None

Disclosures:

SARAH DOYLE, PHD: No financial relationships to disclose

0299

VEGF-A AND THE NLRP3 INFLAMMASOME IN AGE-RELATED MACULAR DEGENERATION

ALEXANDER MARNEROS

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Age-related macular degeneration (AMD) has been traditionally categorized into nonexudative AMD with progressive degeneration of the RPE and retina, and neovascular AMD, where choroidal neovascularization (CNV) impairs vision. Non-exudative AMD can progress to neovascular AMD, and genetic association studies have identified genes that are linked to both forms of AMD, including the gene for VEGF-A. Here, it is shown that mice with increased VEGF-A in all VEGF-A-expressing cells (VEGF-A^{hyper} mice) develop progressive age-dependent cardinal features of both neovascular and nonexudative AMD, suggesting that both forms of AMD may be distinct manifestations of a common underlying process of VEGF-A dysregulation. The data show that increased VEGF-A induces a breakdown of the RPE barrier as the earliest pathologic manifestation, followed by infiltration of macrophages into the subretinal space and subsequent activation of glia cells to produce proangiogenic factors, including IL-1 β and VEGF-A. This retinal glia cell activation occurs at sites of evolving CNV lesions, suggesting a direct role of activated glia cells in promoting CNV. These changes are accompanied by increased VEGF-A-induced oxidative damage to the RPE and accumulation of complement components (particularly C1q), which likely contribute to NLRP3 inflammasome activation that occurs in an age-dependent manner in the RPE in these mice. Targeting the NLRP3 inflammasome or IL-1 β reduces VEGF-A-induced CNV lesions in VEGF-A^{hyper} mice. The data provide a novel link between increased VEGF-A levels and NLRP3 inflammasome activation in AMD.

IRB Status: Approved

Disclosures:

ALEXANDER MARNEROS, MD, PHD: No financial relationships to disclose

0300

NLRP3 INFLAMMASOME, IL-1 β /IL-18, AND IL-17 IN AGE-RELATED MACULAR DEGENERATION

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NIH / NEI¹; ZHONGSHAN OPHTHALMIC CENTER²

Age-related macular degeneration (AMD) is the leading cause of central irreversible blindness in elderly. Although the etiology and pathogenesis of AMD remain unclear, inflammation and oxidative stress are closely involved. Additionally, pathology has illustrated the critical role of the retinal pigment epithelium (RPE) in AMD. Inflammasomes, large intracellular multiprotein complexes, are activated upon cellular infection or stress that trigger the maturation of proinflammatory cytokines such as interleukin (IL)-1 β and IL-18 to engage innate immune defenses. Recently, activation of the NLRP3 inflammasome, a key mediator of neuroinflammation, has been linked to parainflammation, aging, and AMD. Enhanced expression of NLRP3 has been reported in AMD macular lesions. Under oxidative stress, inflammation, or both stimuli, ARPE-19 cells produce higher levels of NLRP3, Caspase-1, IL-1 β , and IL-18 and illustrate abnormal cellular ultrastructure with mitochondrial degeneration, cytoplasmic vacuoles, and autophagosomes. Knockdown of NLRP3 expression in ARPE-19 cells results in lower expression of Caspase-1, IL-1 β , and IL-18. Both IL-1 β and IL-18 can induce IL-17 production through other inflammatory cells, such as Th17 and $\gamma\delta$ T cells. IL-17 and IL-17RC are also aberrantly expressed in maculae with AMD. *In vitro*, IL-17 induces ARPE-19 cell death characterized by the accumulation of cytoplasmic lipids and autophagosomes. siRNA knockdown of IL17RC can block the subsequent activation of pro-apoptotic Caspase-3 and Caspase-9 in ARPE-19 cells. This intervention rescues RPE and photoreceptors in a MAPK-dependent process. These findings suggest a potential role of the inflammasome NLRP3 - IL-1 β /IL-18 - IL-17 pathway in AMD pathogenesis.

IRB Status: Approved

Disclosures:

CHI-CHAO CHAN, MD: No financial relationships to disclose

0301

THE INFLAMMASOME AS THE INTEGRATOR OF MULTIPLE AMD PATHOGENIC STIMULI

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The scientific literature supports a model of age-related macular degeneration (AMD) development that is tremendously

varied and complex. Laboratories throughout the world have proposed multitudes of potential causative agents for AMD including environmental (e.g. tobacco use, diet, light exposure), cellular (e.g. microglial activation, RPE dysfunction), molecular (e.g. VEGF-A, Alu RNA, amyloid beta, iron overload, retinal autoantibodies) and genetic (e.g. complement factor H) factors. Despite the seemingly countless components at play within the degenerating retina, the etiological processes that ultimately lead to macular degeneration appear to follow a generic, predictable pattern. This suggests that multiple, complexly interrelated or independent pathways converge to ultimately bend the homeostatic balance towards a unique cell fate. Here we present new data in support of the concept that the NLRP3 inflammasome, an innate immune signaling pathway, is such an integrating 'danger sensor' in the RPE during AMD. Within and beyond the eye, numerous AMD-related stimuli promote inflammasome activation including Alu RNA, iron overload, amyloid beta, C1q, sub-lethal membrane attack complex activation, light damage, lipofuscin/A2E, lysosomal destabilization and 4-HNE. We and others have further found evidence of inflammasome signaling in human AMD. Finally, the consequences of inflammasome activation unanimously resemble RPE degeneration in human geographic atrophy. From this collection of data, we propose a new model of RPE degeneration in which multiple promoting agents singularly converge on the NLRP3 inflammasome to promote generic RPE cell death in AMD.

IRB Status: Verified

Disclosures:

BRADLEY GELFAND, PHD: No financial relationships to disclose

O302

NLRP3 INFLAMMASOME SIGNALING IN HUMAN RPE CELLS

ANU KAUPPINEN

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Retinal pigment epithelium (RPE) is a single layer of post-mitotic cells maintaining the overlying photoreceptors, i.e. rods and cones. RPE cells are also responsible for the immune defence of macula, and play a central role in the pathogenesis of age-related macular degeneration (AMD), which is the leading cause of severe vision loss in the elderly. Majority of patients have so called dry (atrophic) AMD, whereas about 10% suffer from wet (exudative) form. In the aging eye, a variety of intrinsic and extrinsic factors contribute to the development of inflammation, which is involved in the pathogenesis of both types of AMD. Inflammasome pathways are central signaling systems of innate immunity. Currently, the best-known inflammasomes have a NOD-like receptor NLRP3 as their sensor component. NLRP3 can recognize a wide variety of danger signals encountered by a cell. Sensing the ligand triggers an assembly of a protein complex, which results into the Caspase-1-mediated maturation and secretion of IL-1 β and/or IL-18. Inflammasomes were found in 2002 and recently, NLRP3 inflammasome signaling has been demonstrated also in RPE cells.

IRB Status: None

Disclosures:

ANU KAUPPINEN, PHD: No financial relationships to disclose

RP08 – AMD Genetics, Pathogenesis, and Stem Cells

O303

INFLAMMASOME IN AMD

JAYAKRISHNA AMBATI

UNIVERSITY OF KENTUCKY

Geographic atrophy - the late stage of "dry" age-related macular degeneration - is characterized by RPE cell degeneration. We have identified that activation of the NLRP3 inflammasome is a convergent response to multiple putative AMD-related RPE cell stressors. Targeting of this inflammasome is potential therapeutic strategy for geographic atrophy.

IRB Status: Approved

Disclosures:

JAYAKRISHNA AMBATI, MD: Patents/Royalties relationship with University of Kentucky; Equity Owner relationship with iVeena

O304

OXIDATIVE STRESS IN STEM CELL-DERIVED RPE CELLS: IMPLICATIONS FOR AMD

DEEPAK LAMBA

BUCK INSTITUTE FOR RESEARCH ON AGING

Age-related macular degeneration is a major health-care issue in the ageing population. Its multi-factorial inheritance combined with lack of natural disease in rodents has made it difficult to understand. This, despite tremendous effort, has resulted in no known effective therapies for the more common dry form of the disease. The discovery of pluripotent stem cells has revolutionized the field of human disease research. Now, we have the ability to potentially study the disease development and progression in a dish. In this talk, we will discuss our efforts to generate a model of chronic stress in retinal pigment epithelial cells to study RPE stress response.

IRB Status: None

Disclosures:

DEEPAK LAMBA, MBBS, PHD: No financial relationships to disclose

O305

GENETICS AND STEM CELL BASED THERAPIES FOR MACULAR DEGENERATION

KANG ZHANG¹, Huimin Cai², Alice Song¹

UNIVERSITY OF CALIFORNIA, SAN DIEGO¹; SICHUAN UNIVERSITY²

Age-related macular degeneration (AMD) is the most common cause of visual impairment of the elderly in the developed countries. AMD is a multi-factorial disease that involves interaction of genetic and environmental influences. Allelic variants of genes encoding members of the alternative complement pathway including CFH, CFI and C3 strongly influence an individual's risk of developing AMD. We and others demonstrated that HTRA1 locus at chromosome 10q26 also strongly impact AMD risk (Yang et al, Science, 2006). We showed that variations in CFH, HTRA1, and C3 contribute to a majority of the genetic risk for AMD and are strongly predictive of advanced AMD and bilaterality (Chen et al, Arch Oph, 2010). Smoking is the strongest identifiable environmental factor that interacts with CFH genotypes to impact AMD risk. Epigenetics contributes to aging and age related diseases (Hannum et al, Mol Cell, 2013). Stem cell research shows great promise in modeling disease in vitro and treating blinding degenerative diseases of the eye including AMD and glaucoma (Zhu et al, Cell Stem Cell, 2010; Li et al, PNAS, 2010; Zhang and Ding, NEJM, 2010). The recent advance in genetics and stem cell therapy of eye will allow identification the high risk patients for customized intervention and treatment in the near future (Zhang et al, Nature Review Drug Discovery, 2012)

IRB Status: Approved

Disclosures:

KANG ZHANG, MD, PHD: No financial relationships to disclose

O306

INTERVENTION OF RETINAL DEGENERATION IN RCS RATS WITH SUBRETINAL TRANSPLANTATION OF ADIPOSE-DERIVED STEM CELLS

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TONGJI UNIVERSITY SCHOOL OF MEDICINE, DEPARTMENT OF OPHTHALMOLOGY OF SHANGHAI TENTH HOSPITAL

Retinal degeneration (RD) is a leading cause of blindness in the world with limited treatment. Stem cell approaches, in recent years, provided new hope for improving degenerative diseases. We, therefore, investigated the effects of adipose derived stem cells (ADSCs) in Royal College of Surgeons (RCS) rats, which have been used to mimic dry form of RD, in order to develop an effective therapy for such diseases. ADSCs were prepared from human discarded fat tissue and labeled with lentivirus-GFP, and then monitored for their vascular endothelial growth factor (VEGF) secretion in vitro and their transdifferentiation into other cell types after transplantation. The visual function of RCS rat was evaluated by ERG. Thickness of outer nuclear layer (ONL) and apoptosis of retinal cells were examined to evaluate ADSCs neuron protective effect. The results clearly showed that ADSCs substantially secreted VEGF in cell culture system. In RCS rats, VEGF expression in the tissue of RPE-Bruch's membrane-choriocapillaris complex (RBCC) sharply decreased in the postnatal 4 weeks. Two weeks after the transplantation, the expressions of ONL cell markers including Rho, Crx and OPN1 were increased and the expression of pro-apoptosis genes like Bax, Bak and Caspase 3 were

decreased in ADSC transplantation group. At the 8 weeks post-transplantation, the thickness of ONL was significantly restored in the central part of retina and the rat visual function was significantly improved. There were fewer apoptotic cells in the transplantation sites as detected by TUNEL assay, when compared to the regions far away from the injection sites. However, we failed to observe the transdifferentiation of transplanted ADSCs to other cell types. In summary, we have confirmed that transplantation of ADSCs into the subretinal space of RCS rats could restore their visual functions and delay the degeneration of retina via the donor cells' anti-apoptosis and VEGF mediated neuroprotection.

IRB Status: Approved

Disclosures:

GUO-TONG GT. XU, MD, PHD: No financial relationships to disclose

RP09 – Generating RPE from Stem Cells

O307

A SYSTEMIC THERAPY FOR DRY AMD USING PROGRAMMED HEMATOPOIETIC STEM CELLS

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INDIANA UNIVERSITY, OPHTHALMOLOGY¹; UNIVERSITY OF FLORIDA, PHARMACOLOGY AND THERAPEUTICS²; UNIVERSITY OF FLORIDA COLLEGE OF MEDICINE, MOLECULAR GENETICS AND MICROBIOLOGY³

While retinal pigment epithelial (RPE) cell transplantation into the subretinal space of patients offered a promising therapeutic approach, outcomes to date have been modest. We have previously shown that systemic delivery of hematopoietic stem cells (HSC) genetically reprogrammed with the RPE differentiation marker, RPE 65, enhances repair of the RPE layer and results in recovery of visual function in an acute mouse model of RPE cell loss. Here, we report the efficacy of programming HSC in the superoxide dismutase 2 knockdown (SOD2 KD) mouse model for dry AMD which generates a chronic defect predominantly in the RPE. Systemic administration of HSC infected with lentivirus expressing human RPE 65 (LV hRPE65) or infected with lentivirus expressing LacZ (LV LacZ) was undertaken at 1, 3 and 6 months following induction of the AMD phenotype with SOD2 KD. Outcomes were assessed by visual function tests, histology and immunohistochemistry. LV hRPE65 provided a >75% efficient transfection of HSC with high levels of RPE65 expression. SOD2 KD mice receiving systemic administration of LV hRPE65 programmed mouse HSC retained visual function, exhibited near normal retinal thickness, precluded microglial activation and prevented degeneration of the retina. This preservation of visual function or histology was not observed by null or LV LacZ programmed HSC. At 1, 3 and 6 months following systemic HSC injections into SOD2 KD mice, photopic and scotopic ERGs were significantly higher only in mice administered LV hRPE65-HSC but not mice receiving control LV or untreated SOD2 KD mice. One month following HSC administration to SOD2 KD mice visual

function reached a level similar to WT mice but this declined by about 20% out to 6 months. In conclusion, successful development of this approach will overcome many of the current limitations of human stem cell therapies for early AMD.

IRB Status: None

Disclosures:

MICHAEL E. BOULTON, PHD: No financial relationships to disclose

O308

DEFINED PRODUCTION OF HUMAN EMBRYONIC STEM CELL-DERIVED RETINAL PIGMENTED EPITHELIAL CELLS AS A CELL-BASED TREATMENT FOR AGE-RELATED MACULAR DEGENERATION

SHERRY HIKITA, Britney Pennington, Dennis Clegg, Lincoln Johnson

NEUROSCIENCE RESEARCH INSTITUTE, UC SANTA BARBARA

Retinal pigmented epithelial (RPE) cells derived from human embryonic stem cells (hESC) may be useful as a cellular therapy for the treatment of Age-related Macular Degeneration (AMD), a leading cause of blindness among the elderly in the western world. For clinical relevance, hESC-RPE will need to be produced in sufficient yields under defined and xeno-free conditions. We have developed a protocol to generate hESC-RPE under defined conditions that may be scaled for cell production and banking under Good Manufacturing Practice conditions. Characterization of these hESC-RPE showed significant similarity with human fetal retinal pigmented epithelial cells (hfRPE) in morphology and pigmentation, gene marker expression and function. hESC-RPE manufactured with this protocol may enable stem cell-based therapies for AMD.

IRB Status: Verified

Disclosures:

SHERRY HIKITA, PHD: No financial relationships to disclose

O309

RETINAL SHEET TRANSPLANTS REPLACE PHOTORECEPTORS AND RESTORE VISION

MAGDALENE SEILER¹, Robert Aramant², Biju Thomas³, Pamela Yang², Norman Radtke⁴, Hans Keirstead²

UC IRVINE, DEPARTMENT OF PHYSICAL MEDICINE AND REHABILITATION, STEM CELL RESEARCH CENTER¹; UC IRVINE, DEPARTMENT OF ANATOMY AND NEUROBIOLOGY, STEM CELL RESEARCH CENTER²; UNIVERSITY OF SOUTHERN CALIFORNIA, OPHTHALMOLOGY³; NORTON AUDUBON HOSPITAL, RETINA VITREOUS RESOURCE CENTER⁴

Retinal sheet transplants replace both photoreceptor and RPE, restore visual responses, and serve as a template to develop transplantable retinal progenitor sheets with RPE from human embryonic stem cells (hESCs). Using a custom-made implantation instrument, sheets of fetal retina without or with its RPE were placed into the subretinal space in several retinal degen-

eration models and in patients (FDA-controlled phase 1 and 2 trials; BB-IND #8354). Such transplants develop morphologically normal photoreceptors in contact with RPE and improve light sensitivity in an area of the superior colliculus (SC) that corresponds to transplant placement. Transplants of neural progenitors (fetal cortex) have no effect. Visually responsive areas in the SC have been traced back to retinal transplant neurons, using trans-synaptic pseudorabies virus, indicating a role of synaptic transplant-host connections in visual improvement. Labelled donor processes make synapses with unlabeled host processes in the host inner plexiform layer. In phase 2 clinical trials, sheet transplants of fetal retina/RPE improved visual acuity (EDTRS) in 7 of 10 patients (6 RP, 4 AMD) after 1 year. 1 RP patient stayed the same, and 2 continued to deteriorate. We are currently working on producing retinal sheets from hESC following the method of Nakano et al. 2012 which will be tested in a newly developed immunodeficient S334ter line 3 rat (strain SD-Foxn1Tg(S334ter)3LavRrrc) that does not reject human tissue. Transplantation of retinal sheets has shown a clear beneficial effect on vision, both in animal models and in patients. Based on these results, we have received an Early Translational grant from the California Institute for Regenerative Medicine (CIRM) to develop human embryonic stem cells (hESCs) into sheets of retinal progenitors and RPE, and demonstrate vision restoration in rat models of retinal degeneration.

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IRB Status: Approved

Disclosures:

MAGDALENE SEILER, PHD: Consultant/Advisor relationship with Ocular Transplantation LLC; Patents/Royalties relationship with Ocular Transplantation LLC

O310

SELECTIVE IMMUNOGENICITY OF IPS-DERIVED CELLS

PETER WESTENSKOW, Martin Friedlander

THE SCRIPPS RESEARCH INSTITUTE

Age-Related Macular Degeneration (AMD) is the leading cause of vision loss in the elderly. Current evidence suggests that stem cell-derived retinal pigment epithelium (RPE) grafts may delay photoreceptor degeneration. However, questions regarding their immunogenicity potential persist, and the reprogramming process to convert somatic to induced pluripotent stem (iPS) cells may actually make them more reactive. To address this question mice with humanized immune systems were generated and fibroblasts were isolated from the same human source materials for iPSCs reprogramming. These iPS cells were either directly injected into the host to generate teratomas, or converted into RPE or smooth muscle cells (SMCs) and characterized. They were then transplanted directly into the muscle or eyes of humanized mice (HuSCID) as "autografts." The grafted regions of the muscle and eyes were examined for T-cell infiltration using immunohistochemistry. Unusually small teratomas formed in HuSCID mice after injecting naïve autologous iPSCs

that were largely devoid of specific cell types including smooth muscle (but they contained RPE cells). While significant T-cell infiltration was observed around allografts, none of the iPSC-RPE lines elicited obvious T-cell infiltration. However, all of the autologous iPSC-derived SMC lines examined elicited significant T-cell infiltration in HuSCID mice. These findings suggested that cells derived from iPSC may elicit selective immunogenicity. While this represents a promising outcome for RPE cell transplantation studies, it also serves as a warning against using other iPSC-derived cells without first doing adequate pre-screening to ensure that they are not immunoreactive.

IRB Status: Verified

Disclosures:

PETER WESTENSKOW, PHD: No financial relationships to disclose

0311

MAKING RPE FROM HIPSC – CONSIDERATIONS FOR DISEASE MODELING

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UNIVERSITY OF WISCONSIN-MADISON, WAISMAN CENTER

Human induced pluripotent stem cells (hiPSCs) offer a unique platform for patient-specific disease modeling. This is of particular relevance to macular diseases, where heterologous cell culture systems and laboratory animal models often do not reproduce critical aspects of human disease. Multiple studies have shown that hiPSC can be consistently differentiated into retinal pigment epithelium (RPE), making hiPSC-RPE a promising cell source for patient-specific disease modeling studies. However, there are a number of criteria that hiPSC-derived RPE need to fulfill to be suitable for disease modeling studies. Specifically, it is important 1) that a sufficient amount of hiPSC-RPE cells can be generated for experimentation and drug testing, 2) that the hiPSC-RPE thus obtained show physical and functional attributes associated with *in vivo* human adult RPE and 3) that the disease-associated cellular phenotype can be recapitulated in an *in vitro* model system consisting of hiPSC-RPE alone. Given that individual hiPSC lines yield RPE with variable efficiency, it is also important to evaluate the physical, molecular and functional properties of hiPSC-RPE from multiple patients and controls as well as multiple clones per patient to establish that the observed pathophysiological changes are attributable to the disease. Another important requirement for disease modeling using hiPSC-RPE is to control for culture age and maturity, as RPE cells display different morphological and functional characteristics at different stages of development. To highlight the importance of the above stated criteria, in this presentation I will discuss the steps involved in 1) generating the first “disease in a dish” model of an inherited macular degeneration, Best disease (BD), 2) using the BD hiPSC-RPE model system to gain insights into common pathophysiological processes affecting RPE and 3) targeting key biological defects in BD hiPSC-RPE using specific drugs.

IRB Status: Approved

Disclosures:

RUCHIRA SINGH, PHD: No financial relationships to disclose

0312

PATIENT-SPECIFIC IPSC-BASED CONFIRMATION OF A PATHOGENIC CRYPTIC SPLICE SITE MUTATION IN RPE65 ENABLES PATIENT ENROLLMENT IN GENE AUGMENTATION TRIAL

BUDD TUCKER, Cathryn Cranston, Kristin Anfinson, Dalyz Ochoa, Robert Mullins, Edwin Stone

STEPHEN A. WYNN INSTITUTE FOR VISION RESEARCH, OPHTHALMOLOGY

Purpose: Although RPE65-associated LCA is one of the few retinal degenerative disorders for which clinical gene transfer trials are currently underway, the ability to enroll patients for RPE65 gene therapy is dependent upon identification of 2 bona fide disease causing mutations. Unfortunately, there are numerous patients with phenotypic RPE65 associated disease for whom this has not been possible. Prior to inclusion in a treatment trial, it is essential to determine whether these patients have 1) non-RPE65 associated disease or 2) mutations in RPE65 for which pathogenicity is difficult to determine, e.g. promoter or intronic mutations. The purpose of this study was to determine if an intronic mutation identified in a patient with presumed RPE65 associated disease was truly pathogenic and thus grounds for inclusion in our current clinical trial.

Methods: Blood samples and skin biopsies were obtained from a patient with presumed RPE65 associated disease. Sanger sequencing of the entire RPE65 gene was performed and Pluripotent iPSCs were produced. Retinal pigmented epithelial (RPE) cells were generated via directed differentiation and RT-PCR, Western blotting, immunocytochemistry and confocal microscopic analysis was performed.

Results: Sequencing of the RPE65 gene in a patient with possible RPE65-associated LCA revealed two mutations: 1) a previously identified disease causing exonic leucine-to-proline variation (L408P), and 2) a previously unidentified single point mutation in intron 3 (IVS3-11) resulting in an A>G transition. Pluripotent iPSC-derived RPE formed characteristic pigmented cuboidal cells positive for markers of RPE. RT-PCR analysis using RNA extracted from patient-specific RPE cells revealed that the identified IVS3-11 variation caused a splicing defect, frame shift and subsequent insertion of a premature stop codon.

Conclusions: Patient-specific iPSC-derived RPE cells were successfully used to confirm the pathogenicity of a newly identified intronic RPE65 mutation. These findings will allow the inclusion of this patient into our ongoing clinical gene therapy trial.

IRB Status: Approved

Disclosures:

BUDD A. TUCKER, PHD: No financial relationships to disclose

Retinal Neuroscience and Development

RN01 – Gene Regulation and Protein Modulation in Retinal Development

0313

CONTROL OF NOTCH SIGNALING, PROGENITOR COMPETENCE AND GLIAL AND AMACRINE CELL DIFFERENTIATION BY LHX2

SETH BLACKSHAW, Jimmy de Melo, Cristina Zibetti

JOHNS HOPKINS UNIVERSITY, SCHOOL OF MEDICINE

We investigated the role of Lhx2, a homeodomain transcription factor expressed in retinal progenitor cells and differentiating Müller glia, in regulating cell specification in postnatal retina. Following on our recent identification of a subset of late-stage progenitors in mouse retina that are selectively competent for generating Müller glia, we observe that Lhx2 is required to drive Notch signaling in both gliogenic and neurogenic progenitors. Selective loss of function of Lhx2 in gliogenic-competent progenitors disrupts generation, differentiation and survival of glial precursors, while Lhx2 loss of function in neurogenic progenitors severely perturbs all amacrine cell development. Lhx2 overexpression inhibits gliogenesis, and induces wide-field amacrine differentiation through a Mash1-dependent pathway. We observe that Lhx2 is an essential feed-forward transcriptional cofactor for glial development induced by gliogenic transcription factors such as Hes5. ChIP-Seq and RNA-Seq analysis reveals that Lhx2 directly activates expression of both neurogenic and gliogenic bHLH factors in neonatal retina, along with many other genes selectively expressed in retinal progenitors. In Müller glial precursors, however, Lhx2 selectively regulates expression of gliogenic factors and glial-specific genes. These findings highlight the diverse yet essential functions of Lhx2 in regulation of retinal development.

IRB Status: None

Disclosures:

SETH BLACKSHAW, PHD: No financial relationships to disclose

0314

NUCLEAR RECEPTORS AND TRANSCRIPTIONAL CONTROL OF RETINAL NEUROGENESIS

DOUGLAS FORREST¹, Hong Liu², Yulong Fu², Anand Swaroop³, Soo-young Kim³, Lily Ng²

NATIONAL INSTITUTES OF HEALTH¹; NATIONAL INSTITUTES OF HEALTH, NIDDK²; NATIONAL INSTITUTES OF HEALTH, NEI³

Retinoid-related orphan nuclear receptor b (encoded by the *Rorb* / *Nr1f2* gene) is prominently expressed in retina. Nuclear receptors act as ligand-regulated transcription factors, although the *Rorb* gene belongs to a sub-group of nuclear receptor genes

that encode receptors with no known physiological ligand. *Rorb* encodes two protein isoforms, RORb1 and RORb2. We found that these isoforms display distinct patterns of expression in the mouse retina. RORb1 is widely expressed in the embryonic retina in both dividing progenitor cells and in post-mitotic precursors of several cell lineages including horizontal and amacrine interneurons and in cone and rod photoreceptors. In contrast, RORb2 is expressed during later development and is restricted to photoreceptors. To determine the functions of the *Rorb* gene, we used targeted mutagenesis to delete RORb1 or RORb2 in mouse models. RORb1-deficiency results in lack of horizontal and amacrine neurons. Evidence reveals that RORb1 cooperates with the forkhead factor Foxn4 to induce Ptf1a, a critical factor that promotes the differentiation of horizontal and amacrine interneurons. Additional studies indicate that both RORb1 and RORb2 participate in the induction of the rod differentiation pathway. In summary, the *Rorb* gene is critical for the differentiation of several specific cell types in the neural retina. The diversity of these functions is conferred in part by the differential expression of RORb1 and RORb2 isoforms.

IRB Status: Approved

Disclosures:

DOUGLAS FORREST, PHD: Grant Support relationship with KaroBio Foundation

0315

ONECUT1 AND ONECUT2 REDUNDANTLY REGULATE EARLY RETINAL CELL FATES IN DEVELOPMENT

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UNIVERSITY AT BUFFALO, OPHTHALMOLOGY

Gene regulation plays essential roles in cell differentiation of the central nervous system, including the retina. Previously, we have shown that *Oncut1* (*Oc1*) and *Oncut2* (*Oc2*), two cut domain transcription factors, are expressed in retinal progenitor cells, developing retinal ganglion cells (RGCs), and horizontal cells (HCs). However, in *Oc1*-null mice we only observed an 80% reduction in HCs, but no apparent defect in other cell types, including RGCs. We postulated that the lack of defects in other cell types in *Oc1*-null retinas was due to redundancy with *Oc2*, since the two factors have very similar expression patterns. To test this, we have generated *Oc2*-null mice and now show that their retinas also only have defects in HCs, with ~50% reduction in their numbers. However, when both *Oc1* and *Oc2* are knocked out, the double-knockout (DKO) retinas demonstrate profound developmental defects, including a complete failure to develop HCs. The DKO retinas are also defective in RGC development, as manifested by less optic fibers, thinner optic nerves, and reduced RGC numbers (by ~30%). In addition, severe defects in cone photoreceptor and starburst amacrine cell development also were observed.

These results indicate that Oc1 and Oc2 act redundantly in regulating the determination of multiple cell fates during the early phase of retinal cell differentiation. Expression profiling by RNA-seq suggests that Onecut factors are not only involved in promoting the early cell fates, but also in prohibiting the late cell fates, thus playing an important role in balancing the production of the early and late retinal cell types.

IRB Status: None

Disclosures:

XIUQIAN MU, MD, PHD: No financial relationships to disclose

O316

FOXN4 AND FOXN4-REGULATED DLL4-NOTCH SIGNALING IN RETINAL CELL DEVELOPMENT

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RUTGERS UNIVERSITY¹; UNIVERSITY COLLEGE LONDON²

The generation of appropriate and diverse neuronal and glial types and subtypes during development constitutes the critical first step toward assembling functional neural circuits. During mammalian retinogenesis, all seven neuronal and glial cell types present in the adult retina are specified from multipotent progenitors by the combined action of various intrinsic and extrinsic factors. Early retinal progenitors give rise to several cell types including ganglion, amacrine, horizontal, cone, and rod cells. It is unknown at present how each of these cell fates is selected from the multiple neuronal fates available to the early progenitor. Utilizing a combination of gene-targeting, bioinformatic and biochemical approaches, we have shown that the *Foxn4* winged-helix/forkhead transcription factor is required not only for selecting the amacrine and horizontal cell fates from early progenitors but also for suppressing the alternative photoreceptor cell fates through activating DLL4-Notch signaling. Gene expression and conditional ablation analyses reveal that *Dll4* is directly activated by *Foxn4* via phylogenetically conserved enhancers and that *Dll4* can partly mediate the *Foxn4* function by serving as a major Notch ligand to expand the progenitor pool and limit photoreceptor production. Activating upstream of *Foxn4*, the Meis1 homeodomain factor binds to *Foxn4* enhancers and is required for activating *Foxn4* expression and specifying horizontal cells. Together, our data define a *Foxn4*-mediated molecular and signaling pathway that underlies the selection of amacrine and horizontal cell fates and suppression of alternative photoreceptor cell fates in early retinal progenitors.

IRB Status: None

Disclosures:

MENGQING XIANG, PHD: No financial relationships to disclose

O317

A ROLE OF THE HOMEOPROTEIN TRANSCRIPTION FACTOR RAX IN POSTNATAL PHOTORECEPTOR DEVELOPMENT

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The homeoprotein transcription factor *Rax* is an essential regulator of eye formation. *Rax*-deficient mouse embryos show a complete loss of the optic vesicle. However, the molecular function of *Rax* in late retinal development remains largely unknown. We previously reported that *Rax* activates the expression of transcription factor *Otx2* in the last cell cycle of retinal progenitors and promotes photoreceptor cell fate in the embryonic retina (Muranishi *et al.*, 2011, J. Neurosci. 31; 16792-16807). In the present study, we examined *Rax* function at postnatal stages. We generated an inducible *Rax* conditional knockout (*Rax* iCKO) mouse line in which *Rax* can be deleted in photoreceptor cells upon tamoxifen administration. In *Rax* iCKO mouse retinas, the expression of some of photoreceptor genes was significantly reduced. To identify genes regulated by *Rax*, we performed genome-wide microarray analysis. These results demonstrate that *Rax* controls the expression of cone and rod photoreceptor genes in the postnatal retina. Our study reveals a functional role of *Rax* *in vivo* in postnatal photoreceptor development.

IRB Status: None

Disclosures:

TAKAHISA FURUKAWA, MD, PHD: No financial relationships to disclose

RN02 – Development of Retinal Circuitry and Synapses

O318

SYNAPTIC CIRCUITRY MEDIATING ROD AND CONE SIGNAL IN THE MAMMALIAN RETINA

SAM WU

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Rod and cone signaling pathways constitute major parallel information channels in the visual system. We have studied rod and cone signal interactions at the photoreceptor, bipolar cell (BC) amacrine cell (AC) and ganglion cell (GC) levels in the mouse and monkey retinas. We took advantages of pathway-specific mutant mice and mice with cell-type-specific fluorescent markers to dissect the rod and cone signaling pathways in BCs, ACs and GCs. We also correlated the BC and AC function and morphology with cell-type-specific markers and determined synaptic mechanisms underlying integration and segregation of rod and cone signals in various types of rod and cone BCs, GABAergic and glycinergic ACs and ON and OFF alpha GCs. Additionally, we studied mechanisms underlying retinal degeneration and neuronal dysfunction in mouse models

for eye diseases, such as glaucoma. In experimental glaucoma mouse models of elevated intracellular pressure, we identified synaptic loci where the earliest retinal dysfunction takes places, designed non-invasive tests for early glaucoma detection.

IRB Status: Not provided

Disclosures:

SAM WU, PHD: No financial relationships to disclose

O319

MATURATION OF SYNAPTIC CIRCUITRY UNDERLYING MOTION DETECTION

WEI WEI

THE UNIVERSITY OF CHICAGO

The direction selective ganglion cell (DSGC) in the retina is strongly activated by motion in its preferred direction, but is suppressed by motion in the opposite, "null" direction. Critical to this direction selectivity is a retinal interneuron, the starburst amacrine cell. The starburst amacrine cell provides both excitatory cholinergic inputs and inhibitory GABAergic inputs to the direction selective ganglion cell. Furthermore, these cholinergic and GABAergic circuits exhibit distinct wiring patterns in the adult and play different roles in motion detection. However, little is known about the developmental mechanisms underlying the maturation of the GABAergic and cholinergic networks. To address this question, we examined the properties of the synapses from SAC-DSGC pairs using electrophysiology and multiphoton imaging in the mouse retina. Our data suggest that maturation of synaptic transmission properties and wiring patterns are distinct for GABAergic and cholinergic synapses, highlighting remarkably specificity of developmental regulation of these two synapse types.

IRB Status: None

Disclosures:

WEI WEI, PHD: No financial relationships to disclose

O320

MECHANISM OF DIFFERENTIAL ADHESION MEDIATED CIRCUIT FORMATION AND SPACING

PETER FUERST

UNIVERSITY OF IDAHO, BIOLOGICAL SCIENCES

Purpose: Development of the retina involves interactions of multiple cell types. These interactions regulate the number of each respective cell type, the lamination and synaptic pairing of cells with the appropriate partners and the maintenance and plasticity of established circuits. We have previously characterized the necessity of the Down syndrome cell adhesion molecule (*Dscam*) in many of these processes. In this study we compliment loss of function models with a gain of function model to better understand the contribution of this protein to retinal development.

Result: We find that in some cell populations *Dscam* regulates stratification of dendrite lamination by restricting the depth within the inner plexiform layer at which cells project dendrites. The developmental origin of these ectopic dendrites was explored and defects in refinement and regulation of dendrite growth were detected. Gain of function analysis was then performed to determine which loss of function phenotypes had gain of function correlates. These experiments revealed that an increase in developmental cell death occurs when the gene is over expressed or ectopically expressed, consistent with loss of function, in which a decrease in developmental cell death is observed. While *Dscam* is required to prevent adhesion, over or ectopically expressing the protein did not result in increased or de novo avoidance, suggesting adhesion observed in the *Dscam* mutant retina may not reflect a role for the protein in avoidance. Lamination and other phenotypes observed in the *Dscam* gain of function retina could be rescued by eliminating developmental cell death, suggesting that regulation of cell death may be the primary function of this protein or that it signals through developmental cell death pathways.

IRB Status: Approved

Disclosures:

PETER FUERST, PHD: No financial relationships to disclose

O321

TRANSCRIPTIONAL CONTROL OF RETINAL AMACRINE SUBTYPE DEVELOPMENT

LIN GAN

UNIVERSITY OF ROCHESTER MEDICAL CENTER

Mosaic spacing and dendritic arborization are crucial processes in the establishment of a functional neural circuitry. By spacing their soma, many retina neurons form regular patterns called mosaic spacing: neurons of the same type often distribute themselves with respect to one another and individual neuron usually repels other same-type neurons to avoid too close through the establishment of exclusion zone. Moreover, the neurites from an individual neuron display self-avoidance properties. Though mosaic spacing and dendritic arborization have been intensively studied, the underlying molecular mechanisms are poorly understood and it is unclear whether transcription factors mediate mosaic spacing and dendritic arborization. Here we show that the cholinergic starburst amacrine cells (SACs) in the ganglion cell layer (GCL) are defective in the spacing of cell bodies and in the arborization of dendrites in the *Barhl2*-null retina. The loss of *Barhl2*, a BAR-homeodomain transcription factor, leads to the clumping of displaced SACs in the GCL. In addition, dendrite neurite arborization and self-avoidance of dendrites of cholinergic SACs in the GCL are disrupted in the *Barhl2*-null retina. Interestingly, the defects of mosaic spacing and dendritic arborization are only detected in the SACs of the GCL but not in SACs of the inner nuclear layer (INL). Our results indicate that mouse *Barhl2* regulates mosaic spacing and dendritic arborization. Furthermore, the clumping of SACs in the GCL but not the INL indicates that the mosaic spacing and neurite arborization of the two SAC mosaics are regulated through different mechanisms.

IRB Status: Approved

Disclosures:

LIN GAN, PHD: No financial relationships to disclose

O322

PATTERNS OF GLUTAMATE RESPONSE IN OFF BIPOLAR CELLS OF RAT RETINA

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UNIVERSIDAD DE VALPARAISO, DEPARTAMENTO DE FISIOLOGIA, CNPC¹; UNIVERSIDAD DE VALPARAISO, DEPARTAMENTO DE NEUROCIENCIAS, CINV²

To date, four morphologically different OFF bipolar cell types have been described in rat retina (#1 to 4). Electrophysiological characterization of these cells is incomplete and is not correlated with morphology for each type. In mouse, a fifth OFF bipolar cell classified as 3b has been characterized by immunohistochemistry. This cell type is PKARII β -positive and HCN4-immunonegative, but morphologically identical to type 3a. However, the glutamate response in 3b has not been characterized and it is unknown whether a homologue cell type exists in other mammalian species. In this work, we used whole-cell patch clamp in acute retinal slices to classify different types of rat OFF bipolar cells by their specific voltage-dependent currents and glutamate responses. GYKI-52466 and SYM2081 were used to isolate responses mediated by AMPA and kainate receptors. The response patterns were correlated with cell morphology by fluorescent tracer injection followed by immunohistochemistry to label HCN4 and PKARII β in type 3 bipolar cells. Our results indicate that rat retina contains two different populations of type 3 bipolar cells, one of which is PKARII β -positive, supporting homology with mouse type 3b. Interestingly, some OFF bipolar cells, especially type 4, present a glutamate response pattern with a fast component, and a slow one, which depend on differential activation of kainate and AMPA receptors, respectively. In conclusion, rat retina contains five different functional types of OFF bipolar cells, whose glutamate response patterns confer a temporal identity to each retinal OFF response channel.

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IRB Status: None

Disclosures:

ALEX VIELMA, PHD: No financial relationships to disclose

RN03 – Mechanisms of Neuroprotection

O323

PROLONGING CONE SURVIVAL IN RETINITIS PIGMENTOSA

CLAUDIO PUNZO

UNIVERSITY OF MASSACHUSETTS MEDICAL SCHOOL

Retinitis pigmentosa (RP) is a form of inherited retinal degeneration, which results in the loss of rods and cones. The disease remains largely untreatable and usually leads to blindness. Interestingly, mutations in rod specific genes cause rod and cone death, while mutations in cone specific genes do not affect rods. Since cones are essential for human vision, it is their loss that leads to blindness. We previously showed that rod mediated cone loss in RP is accompanied by gene expression changes in metabolic genes as well as in genes of the insulin/mTOR pathway, which regulates cell metabolism. Further analyses revealed that cones are nutrient deprived in RP. To test the role of the insulin/mTOR pathway during disease, insulin was administered systemically to retinal degeneration 1 (*rd*¹) mutant mice. Insulin treatment significantly improved cone survival, albeit only for a 4 weeks treatment period. This limited effect was likely due to the feedback loop in the insulin pathway.

To unravel how insulin prolongs cone survival, we have genetically activated the pathway in cones of *rd*¹ mice at two different junction points downstream of the insulin receptor. In contrast to activation of the pathway by insulin, the genetic activation is not refractory to the feedback loop. As a consequence of sustained pathway activity, cone survival is significantly prolonged for several months when compared to insulin administration. Cone survival is promoted mainly by improved glucose and amino acid uptake and improved glucose retention through up-regulation of Hexokinase II in cones. The data suggests that increasing the activity of mTOR is a viable strategy to prolong vision in individuals with RP. It further suggests that cone loss in RP is mainly due to a lack of nutrient availability in cones.

IRB Status: None

Disclosures:

CLAUDIO PUNZO, PHD: No financial relationships to disclose

O324

REPROGRAMMING PHOTORECEPTORS

JOSEPH CORBO

WASHINGTON UNIVERSITY SCHOOL OF MEDICINE

Cellular reprogramming studies have demonstrated remarkable plasticity of adult cell types in vertebrates. In this talk, we present two examples of photoreceptor reprogramming, one in mouse and a second in fish. First, we show that acute knockout of *Nrl* in the adult mouse can convert rod photoreceptors into cone-like cells. This reprogramming results in cellular and functional rescue of retinal degeneration in a mouse model of retinitis pigmentosa,

by making the rods immune to a mutation in a rod-specific gene. Next, we present an example of photoreceptor reprogramming that occurs naturally in migratory fish. When salmon migrate from the open ocean into inland streams, they reprogram their photoreceptor for enhanced sensitivity to their new red-shifted optical environment. This reprogramming is mediated by the expression of an enzyme which converts the visual chromophore retinal into 3,4-didehydroretinal that has red-shifted spectral properties. We have identified the enzyme that mediates this 'rhodopsin-porphyrin' switch, and we propose to co-express it with optogenetic actuators *in vivo* to red-shift their action spectra. In this way, we hope to engineer optogenetic devices that can be controlled with far red light, an approach that offers a number of advantages over conventional optogenetic gene therapy strategies that rely on potentially phototoxic blue-green light.

IRB Status: None

Disclosures:

JOSEPH CORBO, MD, PHD: No financial relationships to disclose

O325

CYTOKINE AND DRUG THERAPIES THAT TARGET MITOCHONDRIA AND ENERGY PRODUCTION TO PREVENT DEATH OF PHOTORECEPTORS AND RPE

JOHN ASH, Lei Xu

UNIVERSITY OF FLORIDA, OPHTHALMOLOGY

Damage to mitochondria is a common mechanism of cell death in inherited retinal degenerations. Therefore, enhancing mitochondrial activity is a promising strategy to induce retinal neuroprotection. We have found that cytokines such as leukemia inhibitory factor (LIF) can protect photoreceptors from light stress and inherited retinal degenerations. In this study we have also determined that LIF can protect RPE from sodium iodate induced cell death. In studying the mechanism for protection we have found that LIF induced expression of a subset of nuclear encoded mitochondrial proteins, suggesting that mitochondria are one target for cytokine-induced protection. To further investigate the role of mitochondria and energy metabolism in neuroprotection we studied the ability of the adenosine-monophosphate-dependent-kinase (AMPK) agonist, metformin, to induce protection of photoreceptors and RPE. Our data show that metformin treatment protects photoreceptors from light damage and inherited retinal degeneration and can protect RPE from sodium iodate induced injury. Metformin treatment increased mitochondrial DNA copy number, and increased mitochondrial gene expression under stress conditions. Our data show that metformin-induced neuroprotection of photoreceptor and RPE by priming cells for survival by increasing cellular metabolism and promoting a robust mitochondrial response to oxidative stress. Our data suggest that metformin, or other drugs targeting AMPK, should be developed to prevent or reduce blindness caused by inherited retinal degenerations and degeneration of cones and RPE during age related macular degeneration.

IRB Status: None

Disclosures:

JOHN ASH, PHD: No financial relationships to disclose

O326

MECHANISMS OF CYTOKINE-MEDIATED NEUROPROTECTION

XIAN-JIE YANG, Kun-Do Rhee, Kevin Chao, Steve Nusinowitz, Dean Bok

JULES STEIN EYE INSTITUTE, UNIVERSITY OF CALIFORNIA, OPHTHALMOLOGY

Ciliary neurotrophic factor (CNTF) acts as a potent neuroprotective agent in a variety of retinal degeneration animal models and has been evaluated in clinical trials for treating retinitis pigmentosa (RP) and for dry age-related macular degeneration (AMD). Recently, encapsulated CNTF-secreting cells have been approved by the FDA in clinical applications for RP and dry AMD. Despite the potential of CNTF as an effective therapeutic agent for different blinding diseases, its mechanisms of action in the retina remain poorly understood. We have studied the CNTF signaling mechanisms in the retina using an *rd*/peripherin mutant mouse, which mimics the condition in a type of autosomal dominant RP in patients. By delivering the same CNTF used in clinical trials via viral vectors, we have shown that constitutive, high-level expression of CNTF prevents photoreceptor death but alters the retinal transcriptome and suppresses visual function. Lower doses of CNTF treatment result in the lengthening of inner and outer segments, correction of opsin mislocalization, and significant morphological improvement of rod photoreceptors. However, despite the rescue of degeneration, visual function improvement is still lacking. By performing retinal cell type specific gene deletions, we have identified the initial targets of CNTF as Müller glial cells. Without a functional cytokine receptor in Müller glia, downstream signaling events and CNTF-induced photoreceptor survival are abolished. Furthermore, we show that although the rod photoreceptors do not directly respond to exogenous CNTF, they also require a functional cytokine receptor for survival. Our molecular genetic analyses further demonstrate that exogenous CNTF triggers a complex signaling cascade among glial cells and photoreceptors. Current analyses are aimed at further dissection of the signaling mechanisms involved in neuronal survival in photoreceptor degeneration models. These studies provide insights relevant to the on-going clinical usage of CNTF as a neuroprotective agent for major retinal degenerative diseases.

IRB Status: None

Disclosures:

XIAN-JIE YANG, PHD: No financial relationships to disclose

O327

MYOCILIN-DEFICIENT MICE ARE PROTECTED FROM NEURONAL DAMAGE IN THE RETINA

MARCUS KOCH¹, Bernd Rosenhammer¹, Sebastian Koschade¹, Barbara Braunger¹, Cornelia Volz², Herbert Jaegle², Ernst Tamm¹

UNIVERSITY OF REGENSBURG, INSTITUTE FOR HUMAN ANATOMY AND EMBRYOLOGY¹; UNIVERSITY MEDICAL CENTER REGENSBURG, CLINIC AND POLYCLINIC FOR OPHTHALMOLOGY²

The purpose was to investigate the role of myocilin in the mouse retina. Myocilin is a secreted glycoprotein of the olfactomedin family whose biological function(s) are still largely unclear.

Myocilin-deficient mice (*Myoc*^{-/-}) and *Myoc*^{-/-}; *βB1-Crystallin-Myocilin* mice with ocular overexpression of myocilin were characterized and analyzed by real-time RT-PCR, semithin sectioning and electroretinography (ERG). Apoptosis of retinal neurons was visualized by TUNEL-labeling and quantified. Western blotting was used to investigate different signaling pathways. Apoptosis of RGC was induced by NMDA-injection and excitotoxicity, while apoptosis of photoreceptors was induced by light damage.

During postnatal synaptogenesis, apoptotic death of retinal neurons was significantly decreased in *Myoc*^{-/-} pups. The decrease resulted in a significantly higher number of retinal ganglion cell (RGC) perikarya and their axons in the optic nerve, as well as in an increased thickness of outer and inner nuclear layer in adult *Myoc*^{-/-} mice compared to wild-types. In contrast, myocilin-deficient mice with simultaneous ectopic overexpression of myocilin from the lens (*Myoc*^{-/-}; *βB1-Crystallin-Myocilin*) did not show differences in retinal structure or developmental apoptosis compared to wild-type mice. In adult mice, apoptotic death of photoreceptors following light damage was markedly reduced in *Myoc*^{-/-} mice. Similarly, apoptosis of retinal ganglion cells after excitotoxic damage following an intravitreal injection of NMDA was attenuated in *Myoc*^{-/-} mice. Both effects were rescued upon simultaneous overexpression of myocilin.

We conclude that Myocilin modulates programmed cell death during retinal development and after retinal injury.

IRB Status: None

Disclosures:

MARCUS KOCH, PHD: No financial relationships to disclose

RN04 – Glial Cells in Retina Regeneration and Repair

0328

MÜLLER GLIA-DERIVED PROGENITORS AND RETINAL REGENERATION

ANDY FISCHER, Donika Gallina

OHIO STATE UNIVERSITY, NEUROSCIENCE

Identification of the signaling pathways that influence the reprogramming of Müller glia to neurogenic retinal progenitors is key harnessing the potential of these cells to regenerate the retina. We find that signaling through the Glucocorticoid Receptor (GCR) has a significant impact upon the ability of Müller glia to become proliferating Müller glia-derived progenitor cells (MGPCs). GCR-signaling is commonly associated with anti-inflammatory responses and GCR agonists are widely used to treat inflammatory diseases of the eye, even though the cellular targets and mechanisms of action in the retina are not well understood. We find that the pattern of GCR expres-

sion in the retina is highly conserved across vertebrate species, including chickens, mice, guinea pigs, dogs and humans. In all of these species we find GCR expressed by the Müller glia. An amino acid-sequence alignment of GCR across different vertebrates, from fish to humans, indicates a high degree (≥87% identity) of sequence conservation. In the chick retina, we find that GCR is expressed by progenitors in the circumferential marginal zone (CMZ) and is up-regulated by Müller glia in acutely damaged retinas. Activation of GCR-signaling inhibits the formation of MGPCs and antagonizes FGF2/MAPK-signaling in the Müller glia. By contrast, we find that inhibition of GCR-signaling stimulates the formation of proliferating MGPCs in damaged retinas, and enhances the neuronal differentiation of MGPC progeny while diminishing glial differentiation. Given the conserved pattern of expression of GCR in different vertebrates, we propose that the functions and mechanisms that regulate the expression of GCR are highly conserved and are mediated through the Müller glia. We conclude that GCR-signaling directly inhibits the formation of MGPCs, at least in part, by counteracting the effects of FGF2/MAPK-signaling.

IRB Status: None

Disclosures:

ANDY FISCHER, PHD: No financial relationships to disclose

0329

REPROGRAMMING ZEBRAFISH MÜLLER GLIA FOR RETINAL REPAIR

DANIEL GOLDMAN, Jin Wan, Xiao-Feng Zhao

UNIVERSITY OF MICHIGAN

Disease or injury to the mammalian retina often leads to gliosis and irreparable vision loss. In contrast, the zebrafish retina responds to injury by mounting a robust regenerative response that restores lost sight. Key to this regenerative response are Müller glia that respond to retinal injury by undergoing a reprogramming event so they acquire retinal stem cell characteristics. These reprogrammed Müller glia generate a proliferating population of retinal progenitors that regenerate all major retinal cell types. Understanding the mechanisms by which zebrafish Müller glia are able to reprogram and achieve stem cell characteristics may suggest novel strategies for stimulating Müller glia reprogramming and retinal repair in mammals. Towards this goal we have been investigating mechanisms underlying injury-dependent Müller glia reprogramming in zebrafish. These studies have identified epigenetic events, signal transduction cascades and gene expression programs that drive Müller glia reprogramming, proliferation and neuronal regeneration. An analysis of signaling cascades underlying Müller glia reprogramming and retina regeneration suggests extensive crosstalk at both the signaling and gene expression level. These studies provide a fish-eye's view of the reprogramming and regeneration process and suggest novel strategies for stimulating Müller glia reprogramming in mammals.

IRB Status: Verified

Disclosures:

DANIEL GOLDMAN, PHD: No financial relationships to disclose

O330

EXPRESSING TNFA AND REPRESSING NOTCH SIGNALING ARE NECESSARY AND SUFFICIENT TO INDUCE MÜLLER GLIA DEDIFFERENTIATION AND PROLIFERATION IN THE ZEBRAFISH RETINA

DAVID HYDE, Clay Conner, Kristin Ackerman, Manuela Lahne, Joshua Hobgood

UNIVERSITY OF NOTRE DAME, BIOLOGICAL SCIENCES

Retinal damage in teleosts, unlike mammals, induces robust Müller glia-mediated regeneration of lost neurons. We examined what signals are necessary to initiate Müller glia dedifferentiation and reentry into the cell cycle. We demonstrate that tumor necrosis factor alpha (TNFα) is necessary and sufficient to induce Müller glia proliferation through a Stat3-Jak1 signaling pathway. Additionally, we demonstrate that Notch signaling acts as a negative regulator of Müller glia dedifferentiation/proliferation in the adult zebrafish retina to maintain the Müller glia in a quiescent state in the undamaged retina. Repressing Notch signaling, through injection of the gamma-secretase inhibitor RO4929097, is sufficient to stimulate a subset of Müller glia to dedifferentiate and reenter the cell cycle without retinal damage. This RO4929097-induced Müller glia proliferation is mediated by repressing Notch signaling because inducible expression of the Notch Intracellular Domain (NICD) can repress this effect. The RO4929097-induced proliferation requires Ascl1a expression and Jak1-mediated Stat3 phosphorylation/activation, analogous to the light-damaged retina. While both TNFα and repressing Notch signaling are sufficient to stimulate some Müller glia to dedifferentiate and proliferate, coinjecting RO4929097 and TNFα induced the majority of Müller glia to reenter the cell cycle and produce proliferating neuronal progenitors that committed to a neuronal lineage in the undamaged retina. This demonstrates that removing the Notch inhibitory signal and expressing TNFα are sufficient to induce Müller glia and neuronal progenitor cell responses that mimic those observed in the regenerating retina, which suggests that these two signals represent the majority, if not all, of the signals necessary to initiate Müller glia dedifferentiation and proliferation in the damaged zebrafish retina.

IRB Status: Approved

Disclosures:

DAVID HYDE, PHD: No financial relationships to disclose

O331

CHARACTERIZATION OF SONIC HEDGEHOG FUNCTION DURING MÜLLER GLIA-MEDIATED RETINAL REGENERATION IN ADULT ZEBRAFISH

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WAYNE STATE UNIVERSITY SCHOOL OF MEDICINE

In contrast to the mammalian retina, the zebrafish retina possesses the remarkable ability to regenerate following damage. It is well established that this is primarily accomplished through Müller glial cells. Upon damage, Müller glial cells re-enter

the cell cycle to form progenitor cells, which then migrate to the area of damage and differentiate into new neurons. The purpose of this study is to identify the signals that affect Müller glial cell proliferation and subsequent differentiation of retinal progenitors. A recent report implicated Sonic Hedgehog (Shh) signaling in the proliferation of Müller glial-derived progenitors. In order to definitively determine the role of Shh signaling during adult retinal regeneration we used gain- and loss- of function techniques and two retinal damage models: constant intense light to specifically photo-ablate photoreceptors, and intravitreal injections of the cytotoxin Ouabain to damage all retinal neurons. Using the light-damage model, we first show that Shh signaling induces Müller glial reactive gliosis, including cell hypertrophy and an up-regulation of glial fibrillary acidic protein. In addition, Shh regulates the percentage of Müller glial cells that re-enter the cell cycle following damage and exhibits neuroprotective effects on both rod and cone photoreceptors. Next, using the Ouabain-damage model, we show that Shh signaling also affects the differentiation of retinal progenitors. An increase in Shh signaling specifically increases the number of amacrine and ganglion cells in the regenerated retina, whereas inhibiting Shh signaling results in fewer amacrine and ganglion cells. This result is consistent with the defined role of Shh during retinal development, when Shh signaling is required for differentiation of amacrine and ganglion cells. Together, these data define the pleiotropic roles of Shh during retinal regeneration and add to the growing list of signaling pathways that regulate the regenerative response of Müller glial cells in the adult zebrafish retina.

IRB Status: None

Disclosures:

RYAN THUMMEL, PHD: No financial relationships to disclose

O332

MOLECULAR AND STRUCTURAL ALTERATIONS OF MÜLLER GLIAL CELLS FROM THE "RD" RETINA

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INSTITUTO DE INVESTIGACIONES BIOQUIMICAS, NEUROBIOLOGY

The molecular bases of retinitis pigmentosa (RP) are well-established; however these diseases have still no treatment. The use of stem cells to regenerate the damaged retinas is being intensely investigated as a promising treatment for RP. Recent work from several laboratories has shown that Müller glial cells (MGC) are stem cells in the eye, which may compensate physiological neuronal loss. In addition, MGC have multiple roles in the eye, providing nutrients and trophic factors essential for photoreceptor survival. Little is known about the involvement of MGC in RP. Using a well-known model of RP, the rd mouse, we here investigated whether in addition to the well-known molecular abnormalities of photoreceptors, MGC also presented structural or molecular changes. To investigate this, we prepared mixed neuro-glial cultures from "rd" and wild type (wt) mice retinas. Nuclear morphology was substantially altered in rd MGC. By day 21, 93% of MGC nuclei in wt cultures showed round, oval; or

irregular shapes and only 7% of them evidenced indentations in their morphology. By contrast, in "rd" cultures MGC nuclei having deep indentations increased to about 20%. Expression of nestin, a stem cell marker, was markedly reduced from about 80% in MGC in wt cultures to nearly 40% in "rd" cultures. The filamentous pattern of nestin was usually lost in "rd" MGC. Noteworthy, while in wt cultures each glial cell supported about 2 photoreceptor progenitors, this number was 3 times higher in "rd" cultures. MGC "overload" with photoreceptor progenitors in "rd" mice might deprive them from essential trophic support and contribute to photoreceptor degeneration.

In conclusion, our results suggest that in addition to the alterations in "rd" photoreceptors, changes in structure of MGC and in their interactions with neurons might also be involved in photoreceptor degeneration.

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IRB Status: None

Disclosures:

LUIS POLITI, PHD: No financial relationships to disclose

RN05 – RGC Development, Survival, and Axonal Guidance

O333

DEVELOPMENT MECHANISMS OF VISUAL CIRCUIT FORMATION

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MCD BIOLOGY, UC SANTA CRUZ

The retina performs a wide range of visual processing, including motion detection, color discrimination, and adaptation to changes in light level. This processing is accomplished by parallel circuits in the retina that are comprised of connections between specific types of the six retinal neuronal classes. At the output of each circuit is a unique type of retinal ganglion cell (RGC). There are ~20 RGC types, and their functional specificity arises from selective synapse formation with the other retinal cell types. The different RGC types also have different functions and send axons to specific sets of visual targets and/or stereotypic sublaminae within a target. It is not known how each RGC type acquires its specific characteristics and maintains them during development, nor is it understood how each type of RGC type contributes to visual processing. This knowledge is crucial if we are to understand the neurological basis of visual perception. Here I will present our recent work that has identified key molecules used to specify RGC fate and function.

IRB Status: Approved

Disclosures:

DAVID FELDHEIM, PHD: No financial relationships to disclose

O334

COMBINATORIAL CODES FOR RETINAL GANGLION CELL DEVELOPMENT

TUDOR BADEA

NATIONAL EYE INSTITUTE / NIH

The mammalian retina consists of about 50 distinct neuronal cell types, which participate in a variety of circuits, resulting in about 20 information channels, conveyed by specific Retinal Ganglion Cell (RGCs) types to the brain. The morphology, physiology, circuit function, and development of these RGC types are active areas of investigation. At the molecular level, it is believed that individual cell types may be defined by the unique expression pattern of sets of molecular markers, which convey specific developmental and functional properties. We have been focusing on the transcriptional code that defines the generation of RGC types. Previous studies have established members of the POU and Lim domain transcription factor families as determinants of RGC development. Our working model is that the diversity of RGC types is generated by partially overlapping expression programs, including transcriptional regulators, cell adhesion molecules, cytoskeletal control elements and other molecular families related to neuronal function. We will present evidence of combinatorial gene expression programs in RGCs expressing (or mutant for) the POU domain transcription factors Brn3a and Brn3b and discuss the implications for RGC type diversification and function.

IRB Status: None

Disclosures:

TUDOR BADEA, MD, MA, PHD: No financial relationships to disclose

O335

GENETIC AND VIRUS-BASED APPROACHES FOR PARSING THE CELL TYPES AND CIRCUITS MEDIATING DISCRETE VISUAL BEHAVIORS

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A major goal of neuroscience is to understand how specific cell types and the circuits they participate in mediate defined aspects of visual perception and behavior. An innate and highly adaptive visual behavior is image stabilization: when the head rotates, the image of the visual world slips across the retina. A dedicated subset of retinal ganglion cells (RGCs) and brainstem visual nuclei that together comprise the "accessory optic system" (AOS) are thought to be critical for generating compensatory eye movements that stabilize the retinal image and thereby optimize visual performance.

What RGC types feed the AOS and how might their unique properties contribute to the specific requirements of the image stabilization system? Recently, we discovered a transgenic mouse line, *Hoxd10-GFP*, in which the RGCs projecting to all

the AOS nuclei are fluorescently labeled. Electrophysiological recordings of Hoxd10-RGCs revealed that they include the three subtypes of On direction-selective RGCs (On-DSGCs): responding to upward-, downward-, or forward-motion. Interestingly, Hoxd10-RGCs also include a subtype of On-Off DSGCs tuned for forward motion.

By combining genetic labeling of these cells (Hoxd10-GFP) with retrograde circuit mapping using modified rabies viruses, we discovered a clear parallel pathway segregation of the On- versus On-Off DSGCs projecting to the AOS: the On-DSGCs project to the brainstem centers involved in horizontal and vertical retinal slip compensation whereas the On-Off DSGCs projected only to AOS nuclei controlling horizontal image stabilization. Moreover, we found that the forward-tuned On-Off DSGCs appear physiologically and molecularly distinct from all previously genetically identified On-Off DSGCs.

Our results reveal that the image stabilization network utilizes directional information both from canonical On-type DSGCs, and a specialized subtype of On-Off DSGC. We are now expanding these sorts of analyses to other parallel visual pathways, and exploring the genetic control over their development and function into unique processing streams.

IRB Status: None

Disclosures:

ONKAR DHANDE, PHD: No financial relationships to disclose

O336

ASTROCYTE PHAGOCYTOSIS OF MYELIN DEBRIS DURING XENOPUS LAEVIS METAMORPHIC OPTIC NERVE SHORTENING

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Astrocytes are closely associated with axons in white matter tracts such as the optic nerve, allowing them to respond to signals derived from myelinated axons. In response to injury or disease, astrocytes become highly reactive and, among other things, dramatically up-regulate their phagocytic machinery. However, axonal injury in the mammalian optic nerve is usually accompanied by infiltration of blood-borne professional phagocytes that clear most debris, raising into question the primary function of the astrocytes' own phagocytic machinery. In order to test whether phagocytosis by astrocytes is principally involved in homeostatic debris clearance during non-pathological conditions, an example of extreme yet non-pathological astrocyte phagocytosis, namely the rapid optic nerve shortening during *Xenopus laevis* metamorphosis, was examined. Quantitative analyses based on electron microscopy revealed that approximately one quarter of all myelin in the optic nerve is in the form of debris at the peak of metamorphosis. At this point, astrocytes are the primary phagocytes and there is no infiltration of monocytes into the optic nerve. Serial electron microscopy reconstructions of these metamorphic optic nerve myelinated axons demonstrate

that astrocytes contact and internalize myelin debris through lamellar processes, internalize some debris by pinocytosis, and can develop lipid droplets following myelin internalization. Interfering with their phagocytic activity through expression in astrocytes of dominant negative transgenes for two receptor pathways, Megf11 or Mfge8, or one downstream effector, Rac1, perturb myelin debris clearance from the optic nerve at metamorphosis leading to more myelin per axon. These studies demonstrate that, at least in the *Xenopus laevis* optic nerve, astrocytes can clear large amounts of debris and, when they do so, they also affect myelinated axon homeostasis. These data raise the exciting possibility that regulating the phagocytic activity of astrocytes in the optic nerve may be used to alter disease course in glaucoma.

IRB Status: None

Disclosures:

NICHOLAS MARSH-ARMSTRONG, PHD: No financial relationships to disclose

O337

TISSUE ENGINEERED CELL DELIVERY VEHICLE FOR TRANSPLANTATION OF RETINAL GANGLION CELLS

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UNIVERSITY OF CALIFORNIA SAN DIEGO, SHILEY EYE CENTER AND INSTITUTE OF ENGINEERING IN MEDICINE¹; SCRIPPS HEALTH AND SCRIPPS RESEARCH INSTITUTE, SHILEY CENTER FOR ORTHOPAEDIC RESEARCH AND EDUCATION²

The inability of the adult mammalian retina to replace or regenerate retinal ganglion cells (RGCs) following injury has lead to the study of cell and tissue engineered cell delivery methods. However when designing these delivery devices, it is not sufficient to merely create a scaffold capable of supporting cell growth, it is also necessary to orient axon growth and cellular organization to mimic that of the nerve fiber and ganglion cell layers. Using an electrospinning (ES) method we have created a biodegradable scaffold which directs axon growth radially, mimicking retinal axon growth towards the optic nerve head. Here we describe a method for immobilizing a linear gradient of the guidance molecule Netrin-1 to increase the polarization of RGCs towards the scaffold center. We have further used a 3D printing method to optimize the cell patterning on the electrospun scaffold. Using this scaffold, we have demonstrated an increased ability to direct transplanted RGC axons towards the optic nerve head when compared to injected cells. Taken together, this represents a significant step towards our goal of creating a cell transplantation device capable of guiding their axons to the optic nerve and their eventual targets in the brain.

IRB Status: None

Disclosures:

KARL KADOR, PHD: No financial relationships to disclose

RN06 – Road to Cure I: Stem Cell and ES/iPS Cell Therapy

O338

REGENERATIVE MEDICAL APPROACHES FOR RETINAL DEGENERATIONS: A ROLE FOR MIRNAS

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UNIVERSITY OF WASHINGTON

Restoring retinal neurons after they have degenerated from disease or trauma is a goal of regenerative medical approaches to the restoration of vision. Cell-therapy generally involves either stimulation of an endogenous source of regeneration, or alternatively, transplanting retinal neurons derived from a donor, or from pluripotent stem cells. There have been major advances in the methods for production of retinal cells from pluripotent stem cells. The various types of retinal neurons are generated during development over a specific sequence, and the retinal progenitor cells generated with current protocols from pluripotent cells progress along timelines similar to those of the fetus in vivo. We have recently focused on the potential for miRNAs to control developmental timing in the retina. We will provide an overview of our recent progress in miRNAs in embryonic stem cell derived retina, and efforts to manipulate miRNAs to control the production of specific retinal cell types. The production and purification of specific types of retinal neurons from the mixed populations generated from the multipotent retinal progenitors should provide more effective strategies for cell replacement therapies.

IRB Status: None

Disclosures:

THOMAS REH, PHD: No financial relationships to disclose

O339

CLINICAL TRIALS OF EMBRYONIC STEM CELL-DERIVED RPE FOR RETINAL DEGENERATION

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KECK SCHOOL OF MEDICINE, USC

The RPE is a major site of pathology in age-related macular degeneration (AMD) and human embryonic stem cells (HESC) represent an essentially unlimited source of RPE for cellular therapy. Subretinal injections of cell suspensions of HESC-derived RPE are currently being evaluated in FDA-approved Phase 1/2 human clinical trials for Stargardt's disease (NCT01625559), and geographic atrophy in patients with AMD (NCT01344993). These trials, sponsored by Advanced Cell Technology (ACT), are open label, multi-center, prospective trials. According to the ACT website (www.advancedcell.com), over 30 patients have been treated in the USA and UK and we look forward to the results of these studies. Others, including "The London Project to Cure Blindness" and our project, "The California Project to Cure Blindness", have taken a different approach and have proposed the use of HESC-derived RPE grown as a highly polarized monolayer in vitro prior to sub-

retinal implantation. These monolayers have morphological, molecular and functional characteristics that are similar to the normal RPE monolayer when grown on non-biodegradable membranes. The "London Project" utilizes a polyester membrane, while "The California Project" utilizes a parylene membrane. "The London Project" sponsored by Pfizer is listed on ClinicalTrials.gov (NCT01691261) and will be enrolling patients with acute, wet AMD and recent rapid visual decline. "The California Project" is in final stages of pre-clinical development and is on track to file an IND with the FDA in 2014 for treatment of AMD subjects with geographic atrophy. Polarized HESC-derived RPE show increased secretion of pigment epithelium-derived factor (PEDF) and increased resistance to oxidative stress in vitro. Preclinical studies in Royal College of Surgeons (RCS) rats using polarized HESC-derived RPE grown on parylene demonstrate the safety and efficacy of this therapy as evaluated by imaging, histology and tests of visual function; no teratomas have been identified.

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IRB Status: None

Disclosures:

DAVID HINTON, MD: Equity Owner relationship with Regenerative Patch Technologies (RPT)

O340

GENERATING RETINAL CELLS FROM HUMAN ESCS AND IPSCS

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UNIVERSITY OF WISCONSIN, WAISMAN CENTER, DEPARTMENT OF OPHTHALMOLOGY AND VISUAL SCIENCES

Human pluripotent stem cells (hPSCs) have made a remarkable impact on science, technology and medicine by providing a potentially unlimited source of human cells for basic research and clinical applications. In recent years, knowledge gained from the study of human embryonic stem cells (hESCs) and mammalian somatic cell reprogramming has led to the routine production of human induced pluripotent stem cells (hiPSCs) in laboratories worldwide. hiPSCs in particular show promise for a wide range of uses, including "disease-in-a-dish" modeling, high throughput drug screening, disease gene discovery, gene therapy testing, and transplantation. For these efforts to succeed, it is critical to develop protocols to target differentiation of hPSCs toward cell type(s) of interest. Ideally, such protocols will be highly reproducible, efficient, cost-effective, and robust enough to generate cells in sufficient numbers and purities to facilitate desired studies. In addition, hPSC progeny should exhibit physical, molecular and functional characteristics that confirm their authenticity. One way to provide authentication is to interrogate the cell differentiation process in vitro and ascertain whether it obeys known principles of cell genesis in the developing vertebrate embryo. To date, multiple methods for producing retinal lineage cells from hESCs and hiPSCs have been described, most of which do mimic normal retinal development to some degree. Under

certain culture conditions, hPSCs form optic vesicle-like structures (OVs), which contain proliferating progenitors capable of yielding all neural retina cell types over time. hPSC-OVs can also be directed to an RPE fate when exposed to appropriate signaling cues. Furthermore, knock down or elimination of key transcription factors expressed in early hPSC-OVs led to perturbations of retinal differentiation similar to those seen in animal models. Such observations imply conserved roles for regulators of retinogenesis in hPSC-derived cultures and the developing embryo, and support the *bona fide* nature of hPSC-OV-derived retinal progeny.

IRB Status: Approved

Disclosures:

DAVID GAMM, MD, PHD: No financial relationships to disclose

O341

PHOTORECEPTOR TRANSPLANTATION: ROAD MAP FOR THERAPY

ROBIN ALI

UCL INSTITUTE OF OPHTHALMOLOGY

Retinal degenerations leading to loss of photoreceptors are a major cause of untreatable blindness. Inherited retinal dystrophies affect 1 in 3,000 of the population, and age-related macular degeneration (AMD) affects 1 in 10 people over 60 years. Currently no treatments restore lost photoreceptor cells and visual function and thus there is a need for new therapeutic approaches. We have previously discovered that transplantation of photoreceptor precursor cells at a specific stage of development results in their integration into the adult retina and can improve vision in mice with visual deficits. We have recently shown effective transplantation of mouse embryonic stem cell-derived photoreceptors (Gonzales-Cordero et al., *Nature Biotech*, 2013) and are now developing similar approaches using human embryonic stem cells.

IRB Status: Approved

Disclosures:

ROBIN ALI, PHD: No financial relationships to disclose

RN07 – Cell-to-Cell Signaling and Retinal Development

O342

VASCULAR ENDOTHELIAL CELLS ARE REQUIRED FOR NORMAL RETINAL NEUROGENESIS IN THE ZEBRAFISH

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UNIVERSITY OF IDAHO, BIOLOGICAL SCIENCES¹; HEBREW UNIVERSITY OF JERUSALEM, FACULTY OF MEDICINE²

The roles of the early ocular vasculature in regulating the activities of retinal progenitors are not known. In this study we test

two hypotheses: 1) that local factors provided by the endothelial cells of the ocular vasculature are required for retinal neurogenesis; and 2) that systemic factors from the circulation are required for retinal neurogenesis. To test the first hypothesis, we examined retinal phenotypes in *cloche* (*m39*) mutant zebrafish, which lack hematopoietic cells, endocardial cells, and endothelial cells, and in wild-type zebrafish treated with drugs that inhibit VEGF signaling and therefore lack blood vessels. In addition, we mated *cdh5:gal4* transgenic fish to *UAS:ntr-mCherry* transgenic fish, and treated embryos from these crosses with metronidazole to selectively ablate endothelial cells. To test the second hypothesis, we examined retinal phenotypes in *silent heart* (*troponin T2a*) mutants, which have a non-contractile heart and do not circulate material through their blood vessels, and in *vlad tepes* mutants that fail to develop the erythrocyte blood cell lineage. We observed profound and similar retinal phenotypes in *cloche* mutant embryos, wild-type embryos treated with VEGF signaling inhibitors, and in metronidazole-treated *cdh5:gal4;UAS:ntr-mCherry* transgenic embryos. All of these embryos showed reduced eye size, lens abnormalities, retinal disorganization, reduced retinal cell differentiation, increased cell stress and cell death. However, these phenotypes were not related to hypoxia or the absence of microglia, suggesting a specific developmental function for vascular endothelial cells in regulating retinal neurogenesis. In contrast, the *silent heart* mutants showed only the lens abnormality phenotype, and the *vlad tepes* mutants showed normal lenses and differentiation of retinal neurons. These results suggest that circulating factors are not required for normal retinal neurogenesis, but that circulating factors other than erythrocytes are important for lens development.

IRB Status: None

Disclosures:

DEBORAH STENKAMP, PHD: No financial relationships to disclose

O343

INTERCELLULAR SIGNALING AND MAINTENANCE OF MORPHOGEN RESPONSIVENESS IN THE DEVELOPING RETINA

VALERIE WALLACE

TORONTO WESTERN RESEARCH INSTITUTE

During retinal histogenesis neurons and Müller glia are generated in a temporal sequence. This process is regulated by a combination of cell intrinsic determinants and responses to environmental cues. However, how they are integrated at a molecular level is not well understood. The onset of retinal neurogenesis is marked by differentiation of ganglion cells (GCs), which in turn produce Sonic hedgehog (Shh), a secreted factor, which signals to retinal progenitor cells (RPCs). Shh is an essential morphogen and growth control factor in embryonic development and it functions throughout the period of retinal histogenesis to control cell fate and cell cycle progression and to maintain the progenitor pool. The transcriptional response to Hh is mediated by the Gli transcription factors. We have shown that Gli2 is a key mediator of the Hh response in RPCs and that Gli2 transcription is tightly coupled to differentiation, being rapidly

downregulated in postmitotic cells. Here we have identified a role for intercellular signaling in RPCs for the maintenance of Gli2 expression and competence to respond to Hh signaling.

IRB Status: None

Disclosures:

VALERIE WALLACE, PHD: No financial relationships to disclose

O344

RELATIONSHIPS BETWEEN ORGANELLE DYNAMICS AND POLARIZED SIGNALING DURING RETINAL DEVELOPMENT

BRIAN LINK

MEDICAL COLLEGE OF WISCONSIN

During retinal development, multiple signaling pathways cooperate to influence cell-type fate and morphogenesis. Often, these pathways are coordinated through basic cellular processes. Using genetics, dynamic signal pathway reporters and time-lapse imaging, we have studied how fundamental cellular processes such as interkinetic nuclear migration and endocytosis shape signaling and influence retinal development. Special emphasis will be placed on the Notch, Wnt-betaCatenin, and Hippo-Yap/Taz pathways and their impact on retinal pigment epithelium development and retinal neurogenesis.

IRB Status: None

Disclosures:

BRIAN LINK, PHD: No financial relationships to disclose

O345

ROLES OF WNT SIGNALING DURING EYE DEVELOPMENT

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During fetal development, the formation of the neural retina, retinal pigment epithelium (RPE) and anterior eye segment require highly coordinated tissue-tissue interactions and complex patterning events. Excellent candidates for molecular signals that mediate these processes are the Wnt family members of glycoproteins. Wnts control key processes during development and disease such as proliferation, cell fate, tissue polarity and regeneration. Wnt secretion requires posttranslational modification mediated by the O-acyltransferase Porcupine (Porcn). PORCN deficiency in humans causes focal dermal hypoplasia (FDH, Goltz Syndrome), an X-linked dominant multisystem birth defect that is frequently accompanied with ocular developmental abnormalities resulting in coloboma, microphthalmia or, in severe cases, anophthalmia. To investigate the role of Porcn during mouse eye development, we conditionally disrupted the Porcn gene in diverse ocular and extraocular tissues. We observed that the timing of Porcn disruption dictates the severity

of ocular malformations. Loss of Porcn during morphogenesis of the optic cup in retina, RPE, or extraocular mesenchyme causes mild forms of iris hypoplasia, which occurs more frequently when disrupted simultaneously in multiple tissues. Earlier, at the optic vesicle stage, Porcn depletion in retina, RPE and neural crest-derived mesenchyme frequently results in coloboma and ocular patterning defects. In particular, we observed transdifferentiation of RPE into neural retina, closure defects in the optic fissure and eyelids as well as abnormalities in anterior segment development. Our results suggest that Wnt ligands need to be secreted from multiple tissues at an early stage to achieve a critical threshold of Wnt signaling to regulate distinct processes at different stages during morphogenesis of the eye.

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IRB Status: Approved

Disclosures:

SABINE FUHRMANN, PHD: No financial relationships to disclose

O346

REPROGRAMMING OF THE CHICK RETINAL PIGMENTED EPITHELIUM AFTER RETINAL INJURY

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MIAMI UNIVERSITY AND UNIVERSITY OF DAYTON, BIOLOGY

The embryonic chick can regenerate its retina by the transdifferentiation of the retinal pigmented epithelium (RPE) and by the activation of stem/progenitor cells present in the ciliary margin. These two ways of regeneration occur concomitantly when an external source of fibroblast growth factor 2 (FGF2) is present. During transdifferentiation, the RPE is reprogrammed to become neuroepithelium that can differentiate into the different cell types of the neural retina. Somatic mammalian cells can be reprogrammed to become induced pluripotent stem cells (iPSC) by ectopic expression of pluripotency inducing factors. However, there is limited information concerning the expression of these factors during natural regenerative processes. The generation of iPSC and tissue regeneration could share similar mechanisms and factors. Herein, we investigate the expression of pluripotency inducing factors in the RPE after retinectomy (injury) and during the process of transdifferentiation. We demonstrate that upon injury, the quiescent (p27Kip1+/BrdU-) RPE cells transiently dedifferentiate to become retina progenitor cells expressing *sox2*, *klf4* and *c-Myc* along with eye field transcriptional factors and display a differential up-regulation of alternative splice variants of *pax6*. This transient reprogramming process is not sustained unless FGF2 is present. We identify Lin-28 as a downstream target of FGF2 during the process of retina regeneration. Moreover, we show that overexpression of Lin-28 after retinectomy is sufficient to induce RPE transdifferentiation in the absence of FGF2.

We propose a novel model in which injury signals initiate RPE

reprogramming, while FGF2 upregulates Lin-28, allowing for RPE transdifferentiation.

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IRB Status: Verified

Disclosures:

KATIA DEL RIO-TSONIS, PHD: No financial relationships to disclose

RN08 – Retinal Degeneration Genetics and Mechanisms

O347

UNDERSTANDING THE MOLECULAR BASIS OF RD BY EXOME ANALYSIS

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The purpose of the studies is to identify the molecular basis of retinal degeneration (RD) in patients and families with early onset recessive RD. Mutations in more than 250 genes have been reported to be associated with RD. We have designed probes that selectively capture the exomes of known RD genes, genes listed in cilia proteome, and 13K mapping SNPs to efficiently screen for mutations in known RD and ciliome genes. After known genes have been ruled out, homozygosity mapping can be performed using the custom designed probes to identify regions where novel RD genes may reside. In addition, we have developed software named exomeSuite that analyzes exome data from single individuals, families with multiple members, large cohorts consisting of multiple pedigrees or a group of unrelated individuals by filtering candidate variants based on user specified criteria. Exomes of members of 43 pedigrees were sequenced using the custom capture probes or Agilent V5+UTR kits and analyzed with the exomeSuite software. The causative mutations were identified in 26 of these pedigrees. Four pedigrees had previously reported mutations while 22 had novel mutations in known genes and two pedigrees had potentially damaging variants in novel genes segregating with disease. Involvement of known RD genes has been excluded in 15 pedigrees suggesting the involvement of novel genes in causing RD in these pedigrees.

IRB Status: Approved

Disclosures:

RADHA AYYAGARI, PHD: No financial relationships to disclose

O348

HIGHLY PENETRANT ALLELES IN AGE-RELATED MACULAR DEGENERATION

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Age-related macular degeneration (AMD) is a complex disease caused by a combination of genetic and environmental factors. Genome-wide association studies have identified several common genetic variants associated with AMD, which together account for 15-65% of the heritability of AMD. Multiple hypotheses to clarify the unexplained portion of genetic variance have been proposed, such as gene-gene interactions, gene-environment interactions, structural variations, epigenetics, and rare variants. Several studies support a role for rare variants with large effect sizes in the pathogenesis of AMD. This presentation will provide an overview of the methods that can be used to detect rare variants in common disease, and will review the recent progress that has been made in the identification of rare variants in AMD. In addition, the relevance of these rare variants for diagnosis, prognosis and treatment of AMD will be highlighted.

IRB Status: None

Disclosures:

ANNEKE DEN HOLLANDER, PHD: No financial relationships to disclose

O349

COMPREHENSIVE GENETIC ANALYSIS IN INHERITED RETINAL DISEASES APPLYING NEXT-GENERATION SEQUENCING

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INSTITUT DE LA VISION, PARIS, FRANCE¹; INSERM-DHOS CIC1423²

Inherited retinal disorders (IRD) are clinically and genetically heterogeneous with more than 150 implicated gene defects and several others to be discovered. Over the past two decades, approaches including linkage analysis, Sanger sequencing, chip technology and homozygosity mapping were used to investigate the genetics of IRD. Despite many advantages, the above-mentioned technologies have limitations on diagnostic value, availability of suitable families or enormous efforts in terms of time and cost. Unbiased novel sequencing techniques provide accurate information about any type of nucleic acid in a given sample at a high throughput while incurring relatively limited costs. We have previously developed a retinal gene panel applying this next generation sequencing (NGS) technique, which was subsequently validated and enhanced to improve the coverage of targeted genomic regions and restricted to the most relevant genes underlying progressive inherited retinal disorders. Herein, we present different filtering approaches that we applied to identify the genetic

defects and their prevalence in a French IRD cohort using this panel and whole exome sequencing (WES). This work resulted recently in the identification of a gene underlying autosomal recessive rod-cone dystrophy (RCD), *KIZ*, coding for the Kizuna centrosomal protein. In total 3 different truncating mutations in more than 340 patients have been identified. Analysis in mice detected *Kiz* mRNA levels in rod photoreceptors, with its decreased expression along with photoreceptor degeneration in *rd1* mice. The presence of human *KIZ* transcript was confirmed by quantitative RT-PCR in retina, retinal pigment epithelium, fibroblast and whole blood cells, with highest expression in retina. RNA *in situ* hybridization demonstrated the presence of *Kiz* mRNA in the outer nuclear layer of mouse retina. Immunohistology revealed KIZ localization at the basal body of the cilia in human fibroblasts thus shedding light on another ciliary protein implicated in autosomal recessive RCD.

IRB Status: Approved

Disclosures:

CHRISTINA ZEITZ, PHD: No financial relationships to disclose

O350

CHARACTERIZATION OF NOVEL GENES RESPONSIBLE FOR HEREDITARY RETINAL DISEASES IN JAPANESE POPULATION

TAKESHI IWATA

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NATIONAL INSTITUTE OF SENSORY ORGANS

The whole exome analysis was performed for Japanese patients with hereditary retinal diseases for the first time. Total of 496 affected families with hereditary retinal disease including retinitis pigmentosa (RP), Leber's congenital amaurosis (LCA), macular dystrophies (MD), cone/rod dystrophy (CRD), stationary night blindness (SNB) and others were collected for clinical information and DNA samples to the National Institute of Sensory Organs. Approximately 500 DNA samples from 208 RP, 64 OMD, 23 LCA, 26 STGD, 42 CRD, 17 SNB and 34 MD pedigrees were analyzed by whole exome sequencing using SureSelect XT Human All Exon kit V4 + UTRs kit (Agilent Technologies) and HiSeq2000 sequencer (Illumina) for 100-bp paired-end sequencing. Reads were mapped to the reference human genome (1,000 genomes phase 2 reference, hs37d5) with Burrows-Wheeler Aligner (BWA) software ver. 0.6.2. Duplicated reads were then removed by Picard MarkDuplicates module ver. 1.62, and mapped reads around insertion-deletion polymorphisms (INDELs) were realigned by using the Genome Analysis Toolkit (GATK) ver. 2.1-13. Calling of mutations was performed by using the GATK UnifiedGenotyper module, and called single-nucleotide variants (SNVs) and INDELs were annotated by using snpEff software ver. 3.0. The mutations were then filtered so that only those with "HIGH" or "MODERATE" snpEff scores and a frequency of less than 1% in the 1,000 genome database and the 1,500 Japanese exome database was further analyzed. The analysis resulted with only 17% of affected samples detected with known mutations. Novel gene mutations in known gene were detected in 14% of the pedigrees.

These genes include EYS and CNGA1 for ARRP and PRE65, CRB1, RDH12 for LCA. Novel gene mutations were found in 8% of the pedigrees. The other 61% is currently under investigation. These novel mutations are likely to be common in Asian populations.

IRB Status: International

Disclosures:

TAKESHI IWATA, PHD: No financial relationships to disclose

O351

MUTATIONS IN COL4A1 CAUSE RETINAL VASCULAR LESIONS

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Type IV collagen alpha 1 (COL4A1) is a major component of almost all basement membranes, and mutations in *COL4A1* cause multisystem disorders in humans and mice. Notably, patients and mice with *Col4a1* mutations exhibit various ocular defects including Anterior Segment Dysgenesis, Optic Nerve Hypoplasia and retinal vascular tortuosity. Here we examine mice carrying a dominant-negative *Col4a1* mutation (*Col4a1^{Δex41}*) for retinal phenotype.

We examined retinas of *Col4a1^{+/-Δex41}* C57BL/6J.129S1/SvImJ mice and *Col4a1^{+/-}* littermate controls at different ages from post-natal day (P) 21 to P720 *in vivo* using fluorescein angiography (FA), funduscopy and optical coherence tomography. Histology, immunohistochemistry and quantitative PCR analyses complemented our analyses.

FA showed patterns of retinal tortuosity and ramification in all *Col4a1^{+/-Δex41}* animals. Retinal examinations revealed serous chorioretinopathy, retinal hemorrhages, fibrosis or signs of pathogenic angiogenesis with anastomosis of the choroid and the retinal vasculature in approximately 80% of all *Col4a1^{+/-Δex41}* eyes at various ages. Retinal hemorrhages and anastomosis were observed as early as P21. We assessed expression levels of candidate genes involved in angiogenesis and found increased vascular endothelial growth factor (VEGF) expression in *Col4a1^{+/-Δex41}* retinas compared to *Col4a1^{+/-}* retinas.

Our findings suggest that patients carrying mutations in *Col4a1* may be at risk for sudden vision loss resulting from retinal vascular insults. Elevated VEGF expression may be responsible for the vascular lesions or may be a consequence that then leads to further retinal lesions. Currently VEGF is a major target for preservation of vision in patients with age-related macular degeneration. This suggests that anti VEGF therapy may also be an effective strategy to prevent pathogenic vascular defects that result from *COL4A1* mutations.

IRB Status: Approved

Disclosures:

MARCEL ALAVI, PhD: No financial relationships to disclose

RN09 – Epigenetics in Development and Diseases

O352

POLYCOMB REGULATION OF RETINAL PROGENITOR PROLIFERATION AND DIFFERENTIATION

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The histone methyltransferase complex PRC2 is a repressive complex that controls key steps in developmental transitions and cell fate choices, but much remains to be learned about its roles in vertebrate eye development. We have investigated PRC2 function in both *Xenopus* and mouse eye development. In both species we found that the PRC2 core components are enriched in retinal progenitors and downregulated in differentiated cells. In *Xenopus*, morpholino knockdown of the PRC2 core component Ezh2 caused reduced retinal progenitor proliferation, in part due to upregulation of the Cdk inhibitor p15(Ink4b). In addition, PRC2 knockdown caused suppression of proneural bHLH gene expression, resulting in reduced retinal neuron differentiation and an increase in Müller glial cell differentiation. In mouse, conditional knockout of Ezh2 resulted in premature cell cycle exit of retinal progenitors at early postnatal stages, resulting in disruption of postnatal retinal histogenesis and lamination. Loss of Ezh2 was linked to aberrant upregulation of multiple genes, including the Cdk inhibitor p16 (CDKN2A). We conclude that in both *Xenopus* and mouse, PRC2 function is required to maintain retinal progenitor proliferation and the correct program of retinal gene expression.

IRB Status: Verified

Disclosures:

MONICA VETTER, PHD: No financial relationships to disclose

O353

3-D CHROMATIN ORGANIZATION OF MURINE PHOTORECEPTORS

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WASHINGTON UNIVERSITY SCHOOL OF MEDICINE, OPHTHALMOLOGY AND VISUAL SCIENCES¹; WASHINGTON UNIVERSITY SCHOOL OF MEDICINE, MOLECULAR GENETICS AND GENOMICS PHD PROGRAM²

Rods and cones arise from a common precursor but express distinct sets of genes. Using Chromosomal Conformation Capture (3C) assays with *opsin* genes as models, we previously showed that the chromatin of each *opsin* locus undergoes cell-type-specific organization, with regulatory regions looping to interact with coding regions (*cis* interactions). Here we further investigate how this chromatin organization is established during development, and whether these regulatory loops interact with other transcribed gene loci via *trans* chromosome interactions. Circularized Chromosome Conformation Capture-Sequencing

(4C-seq) was performed on FACS-sorted rod and cone photoreceptors to determine genome-wide chromatin interactomes of 20 differentially expressed loci. The results confirmed the *cis* loop interactions between the regulatory and coding regions of actively transcribed *opsin* gene(s). 4C-seq also identified novel interactions of *opsin* regulatory regions with a number of other regions in the same and on different chromosomes, revealing potential novel enhancers even several Mb away from active genes, and unique chromosomal links between co-regulated genes. The biological significance of the complex *cis* and *trans* interactions of these gene loci are being approached by reviewing the 4C-seq data in the context of published datasets describing other epigenetic phenomena, such as DNase I hypersensitivity (chromatin accessibility), binding profiles of photoreceptor transcription factors, and regulatory histone marks, as well as transcriptional status. This provides a new understanding of the manner by which active and inactive genes are organized in mature photoreceptors and how genes may share transcription-promoting factors and machinery. Together, our findings suggest a dynamic and regulated organization of photoreceptor genomes, providing a new level of epigenetic regulation of photoreceptor gene expression.

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IRB Status: Approved

Disclosures:

SHIMING CHEN, PHD: No financial relationships to disclose

O354

ON THE VERGE OF NEURONAL REPLACEMENT: CELLULAR PLIANCY AND RETHINKING AGE-OLD DOGMA

MIKE DYER

ST. JUDE CHILDREN'S RESEARCH HOSPITAL, DIVISION OF DEVELOPMENTAL BIOLOGY

My lab is interested in understanding how proliferation and differentiation are coordinated in the developing retina and how those processes become uncoupled in retinoblastoma. Recently, we made a discovery that has fundamentally altered our understanding of the molecular and cellular mechanisms of retinal development and may also have a major impact on efforts to restore vision in some patients with retinal degeneration. We discovered that individual retinoblastoma tumor cells express multiple developmental programs simultaneously. This occurs through deregulation of the epigenetic programs that are directly or indirectly regulated by the RB1 protein. To explore this finding further, we developed a novel experimental system to quantify the epigenetic reprogramming of individual retinal neurons by using 4 factors (Oct4, Klf4, Sox2, and Myc) and somatic cell nuclear transfer. We discovered that the epigenetic barriers to reprogramming dramatically differ across retinal cell types, and they are developmental stage-specific. Moreover, we have used a 3-dimensional culture system to show for the first time that mouse iPSCs can form the optic cup and differentiated retinæ. One of the most exciting results from these experiments is that our iPSC lines derived

from retinal neurons are more efficient at differentiate into laminated retinae than fibroblast derived iPSCs or embryonic stem cells. Our current research is focused on elucidating the underlying molecular mechanisms that contribute to this epigenetic memory of retinal derived iPSCs. It may also provide crucial preclinical data on the use of retinal-derived iPSCs for future clinical trials of photoreceptor-replacement therapy to treat retinal degeneration.

IRB Status: Not provided

Disclosures:

MIKE DYER, PHD: No financial relationships to disclose

O355

HYBRID MICE REVEAL PARENT-OF-ORIGIN AND CIS- AND TRANS-REGULATORY EFFECTS IN THE RETINA

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Gene expression is regulated by *cis*-regulatory elements (CREs, i.e., enhancers, promoters, and silencers) and by the *trans* factors (e.g., transcription factors) that act upon them. Photoreceptors are photosensitive neurons in the retina and are exquisitely susceptible to genetic disease. The transcriptional networks governing photoreceptor development have been extensively studied, and the regulatory regions of photoreceptor genes have been mapped genome-wide. However, the relative contributions of *cis*-regulatory and *trans*-regulatory effects in the retina are unknown. A powerful approach to dissecting *cis* and *trans* effects is to compare F1 heterozygous hybrids with F0 homozygotes. Taking advantage of the high frequency of polymorphisms in wild-derived inbred Cast/EiJ mice relative to the standard reference strain C57BL/6J, we utilized the F1 hybrid study approach with RNA-seq in the adult mouse retina. We found that *cis* effects accounted for the bulk of gene regulatory divergence between these two strains. Many of the genes subjected to *cis* effects were associated with variants within photoreceptor CREs. A subset of genes was associated with human retinal disease, likely reflecting activity-altering variants with phenotypic consequence. Furthermore, by comparing our retinal data with previously published liver data, we found that most of these *cis* effects were tissue-specific. As part of our analyses, we identified parent-of-origin (e.g., imprinting) effects. We found that many of the strongly imprinted genes in the retina are known imprinted genes based on studies of other tissues. Our study provides a resource for mapping sequence variants onto changes in gene expression and underscores the importance of studying *cis*-regulatory variants in the context of retinal disease.

IRB Status: None

Disclosures:

SUSAN Q. SHEN, BS: No financial relationships to disclose

RN10 – Road to Cure II: Gene Replacement, Optogenetics, and Other Therapies for Retinal Diseases

O356

RESTORATION OF THE MAJORITY OF THE VISUAL SPECTRUM IN RCS RATS USING AAV-MEDIATED MODIFIED VOLVOX CHANNELRHODOPSIN-1 GENE TRANSFER

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IWATE UNIVERSITY, CHEMISTRY AND BIOENGINEERING¹; TOHOKU UNIVERSITY²

We previously showed that blind rats whose vision was restored by gene transfer of *Chlamydomonas* channelrhodopsin-2 (ChR2) could only detect wavelengths less than 540 nm because of the action spectrum of the transgene product. *Volvox*-derived channelrhodopsin, VChR1, has a broader, red-shifted action spectrum that is useful for restoring vision. We initially planned to use VChR1 to increase the wavelength sensitivity for restoring vision and therefore transferred the VChR1 gene into genetically blind (RCS) rats by using an adeno-associated virus vector. However, we could not record any VEPs in the VChR1-transferred RCS rats and found that VChR1 had extremely low plasma membrane integration. The VChR1 protein was mainly localized in the cytoplasm and showed weak ion channel properties when the VChR1 gene was transfected into HEK 293 cells. We generated modified *Volvox* channelrhodopsin-1 (mVChR1), which is a chimera of *Chlamydomonas* channelrhodopsin-1 and *Volvox* channelrhodopsin-1, and demonstrated increased plasma membrane integration and dramatic improvement in its channel properties. Under whole-cell patch clamp, mVChR1-expressing cells showed a photo-induced current upon stimulation at 468–640 nm. The evoked currents in mVChR1-expressing cells were approximately 30 times larger than those in VChR1-expressing cells. RCS rats expressing mVChR1 via an adeno-associated virus vector regained their visual responses to light with wavelengths between 468 and 640 nm and their recovered visual responses were maintained for a year. Thus, mVChR1 is a candidate gene for gene therapy for restoring vision, and gene delivery of mVChR1 may provide blind patients access to the majority of the visible light spectrum.

IRB Status: International

Disclosures:

HIROSHI TOMITA, PHD: No financial relationships to disclose

O357

RESTORING VISUAL FUNCTION BY ECTOPIC EXPRESSION OF MELANOPSIN TO RETINAL BIPOLAR CELLS IN RETINAL DEGENERATION

BIN LIN

UNIVERSITY OF HONG KONG

Retinitis pigmentosa (RP) is caused by a large number of mutations that result in the loss of photoreceptors and subsequent partial or complete blindness. At present, no effective treatment is available for restoring vision to RP patients once photoreceptors have been lost. To restore photosensitivity to the photoreceptor degenerated retinas, we genetically targeted photosensitive pigment melanopsin to the surviving bipolar cells, the second-order neurons, to convert them into photosensitive photoreceptors in a mouse model of RP. We used an adeno-associated viral vector (AAV) to produce widespread expression of melanopsin in bipolar cells in rd10 mice. We found that ectopic expression of melanopsin in bipolar cells restored light responses in the retina. The treatment also allowed the animals to respond to light and dark and to successfully restore optomotor behavioral performance. The melanopsin-based gene therapy thus has great potential to restore light perception in humans suffering from photoreceptor degenerations.

IRB Status: None

Disclosures:

BIN LIN, PHD: No financial relationships to disclose

O358

RESTORING VISUAL FUNCTION TO BLIND MICE WITH CHEMICAL PHOTOSWITCHES THAT EXPLOIT ELECTROPHYSIOLOGICAL REMODELING OF THE DEGENERATING RETINA

RICHARD KRAMER, Ivan Tochitsky

UNIVERSITY OF CALIFORNIA, BERKELEY

Retinitis pigmentosa (RP) and age related macular degeneration (AMD) are blinding diseases caused by the degeneration of rod and cone photoreceptors in the retina, which leaves the rest of the visual system largely intact but unable to respond to light. Visual responses can be restored in a mouse model of RP, by applying a synthetic ion channel photoswitch compound that confers light-sensitivity onto retinal neurons, downstream from the degenerated rods and cones (Polosukhina et al., 2012). However photosensitization persists for only a few hours and requires very high intensity, near-UV light, unsuitable for clinical application. Here we report red-shifted photoswitch compounds that generate robust responses to light with intensity equivalent to ordinary daylight. A single intravitreal injection can photosensitize the retina for several weeks, restoring electrophysiological and behavioral responses with no apparent toxicity. We compared different strains of mice with rods and cones that were functional, non-functional, or degenerated. We found that the red-shifted photoswitches only affected mice lacking rods and cones, indicating a drug target that is selectively expressed in retinas with degenerative disease. The high light sensitivity, favorable spectral sensitivity, and selective targeting to diseased tissue make these red-shifted photoswitches prime drug candidates for clinical vision restoration in patients with end-stage RP and AMD.

IRB Status: Approved

Disclosures:

RICHARD KRAMER, PHD: Equity Owner relationship with Photoswitch Biosciences, Inc.

O359

EVALUATION AND DEVELOPMENT OF MORE LIGHT-SENSITIVE CHR2 MUTANTS FOR VISUAL RESTORATION

ZHUO-HUA PAN¹, Tushar Ganjawala¹, Qi Lu¹, Elena Ivanova², Zhifei Zhang¹

WAYNE STATE UNIVERSITY¹; BURKE MEDICAL RESEARCH INSTITUTE²

Ectopic expression of microbial opsin-based optogenetic sensors such as channelrhodopsin-2 (ChR2) in surviving inner retinal neurons has been a promising approach to restoring vision after retinal degeneration. A major limitation for using ChR2 as a light sensor for the purpose of vision restoration, however, is its low light-sensitivity. Recently, ChR2 mutations of T159C and L132C, were reported to result in highly or ultra light-sensitive. In this study, we created additional ChR2 mutants at these two sites to search for more light-sensitive ChR2 forms and evaluate their suitability for vision restoration by examining their light response properties along with the previously reported mutants in HEK cells and retinal ganglion cells in mice. We found additional ChR2 mutants at these two sites that show a further increase in operational sensitivity to light. However, the increase in the light sensitivity was correlated with a decrease in temporal kinetics. Therefore, there is always a trade-off between light sensitivity and temporal resolution for these more light-sensitive ChR2 mutants. Our results showed that for the two most light-sensitive mutants, L132C/T159C and L132C/T159S, the required light intensities for generating the threshold spike activities in retinal ganglion cells were about 1.5 and nearly 2 log units lower than that of wt ChR2, while their ChR2-mediated spike activities could still follow the flicker frequencies up to 20 and 10 Hz, respectively. Thus, the use of these more light-sensitive ChR2 mutants could make the optogenetic approach to restoring vision more feasible.

IRB Status: Approved

Disclosures:

ZHUO-HUA PAN, PHD: Patents relationship with Wayne State University; Consultant/Advisor relationship with RetroSense Therapeutics

O360

AN NRF2-DERIVED PEPTIDE DELIVERED BY AN AAV VECTOR PROTECTS THE EYE AGAINST INFLAMMATION AND OXIDATIVE STRESS

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Oxidative stress has been linked to several ocular diseases. This stress may also initiate an inflammatory response that increases both tissue injury and the level of reactive oxygen species. The *Nrf2* gene is a transcription factor known to regulate

the expression of antioxidant genes whose function is tightly regulated by its repressor Keap-1. We have used a viral vector to deliver a small peptide derived from the region of the Nrf2 protein that binds Keap-1. The DNA sequence coding for this peptide was fused to the HIV tat peptide sequence to provide the Nrf2 peptide (Nrf2mer) with cell penetration properties. This TatNrf2mer peptide can be expressed *in vitro* and it induces the expression of antioxidant genes, blocks and protects cells against oxidative stress. This peptide was also found to block the secretion of the pro-inflammatory cytokine IL-1 β . We fused the TatNrf2mer sequence to a secretable GFP (sGFP) which can be proteolytically separated from the peptide upon reaching the cell membrane. The ability of this vector to protect against oxidative stress was studied in the sodium iodate (NaIO₃) mouse model of RPE injury. Mice were injected intravitreally with the AAV vector expressing the sGFP-TatNrf2mer fusion gene and one month later were challenged with NaIO₃. Seven days later

we found protection of the ERG a- and b-wave responses in the eyes treated with the sGFP-TatNrf2mer AAV vector when compared to the control treated eyes. We also tested this vector in the endotoxin-induced uveitis mouse model. The expression of sGFP-TatNrf2mer resulted in a 54% decrease in the number of inflammatory cells in the vitreous body when compared to control treated eyes. These results demonstrate that our TatNrf2mer AAV vector has antioxidant and anti-inflammatory effects in widely-used models of ocular injury, suggesting that it could be useful for preventing damage associated with AMD.

IRB Status: None

Disclosures:
CRISTHIAN ILDEFONSO, PHD: No financial relationships to disclose

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Retinal Cell Biology

RC01 – Role of Retinal Clocks in Eye Disorders

O361

CIRCADIAN REGULATION OF RETINAL FUNCTIONS AND PATHOPHYSIOLOGY

GIANLUCA TOSINI

MOREHOUSE SCHOOL OF MEDICINE

Daily rhythms are a ubiquitous feature of living systems. Generally, these rhythms are not just passive consequences of cyclic fluctuations in the environment, but instead originate within the organism. The retina plays a critical role in the organization of the circadian system by synchronizing the brain's central clock with the external day through transduction of the daily light/dark cycle. However, the substantial variation in luminance imposed on the retina between day and night also poses a challenge to its role as a sensory tissue. In this regard, the retina has evolved not only sophisticated mechanisms of light- and dark-adaptation, but its own local circadian clock to allow anticipation of the regular cycle of the solar day. Several studies have shown that melatonin and dopamine are important regulators of retinal physiology and play opposing roles in the regulation of retinal adaptive physiology. DA functions as a humoral signal for light, producing light-adaptive physiology whereas melatonin, on the other hand, has dark-adaptive effects. Thus, the melatonin secreting photoreceptors and DA secreting neurons form an intercellular feedback loop functioning to regulate circadian retinal physiology. Emerging experimental evidence suggests that the retinal molecular clockworks, or its output signals, may also contribute to retinal disease and pathology. Indeed recent studies have shown that removal of melatonin signaling affect photoreceptors and ganglion cells viability and the loss of rhythmicity in retinal pigment epithelium phagocytosis leads to reduced viability of the photoreceptors during aging and mice with a dysfunctional circadian clock develop several pathologies in the retina and in other ocular structures. The symposium on the role of retinal clocks in eye disorders will focus on the role of the circadian clock in the regulation of retinal functions and how alteration of these functions may lead to ocular pathologies.

IRB Status: None

Disclosures:

GIANLUCA TOSINI, PHD: No financial relationships to disclose

O362

RETINAL MÜLLER CELLS ARE CLOCK CELLS AND CLOCK GENES IMPACT RETINAL NEOVASCULARIZATION

DOUGLAS MCMAHON, Lili Xu, John Penn

VANDERBILT UNIVERSITY

Circadian rhythms generation is likely distributed across several cell types in the retina. Here, we have shown that retinal Müller glia express the full complement of core circadian clock genes and exhibit circadian clock function, demonstrating circadian rhythms in bioluminescence from purified mouse Müller cells derived from PER2::LUC circadian reporter mice. Retinal Müller cell cultures exhibit robust freerunning near 24-hour rhythms in gene expression that persist in for several days. These rhythms are inhibited by knockout, or knockdown, of the clock genes *Period1* or *Bmal1*. Human Müller cells transduced with lentiviral circadian gene reporters also exhibit robust rhythms. Given that Müller cells are an important source of vascularizing signals in the retina, and *Period* clock genes have been shown to influence VEGF in tumor cell lines, we examined the potential impact of the *Period1* and *Period 2* on VEGF secretion and retinal neovascularization in a mouse model of oxygen induced retinopathy (OIR). We found that in *Per1/Per2* double knockout mice VEGF secretion was influenced rhythmically by the circadian clock, that peak VEGF levels were increased in OIR in *Per1/2* double knockout retinas vs wt, and that there was an increase in retinal neovascularization in the *Per1/2* double knockout retinas. These results indicate that retina Müller glia are circadian clocks, even when isolated from other retinal cell types, and suggest that clock genes, perhaps in the Müller glia, are important regulators of neovascularization signals.

IRB Status: None

Disclosures:

DOUGLAS MCMAHON, PHD: No financial relationships to disclose

O363

RETINAL GANGLION CELLS IN BIRDS: DAY-TIMERS AND NON-VISUAL PHOTORECEPTORS

MARIO GUIDO, Diego Valdez, Paula Nieto, Maria Contin, Daniela Verra, Nicolas Diaz, Eduardo Garbarino-Pico

UNIVERSIDAD NACIONAL DE CÓRDOBA-CONICET

Chicken retinal ganglion cells (RGCs) contain autonomous oscillators that generate daily rhythms in melatonin synthesis and in the activity of its regulatory enzyme arylalkylamine *N*-acetyltransferase (AA-NAT). An intrinsically photosensitive RGC subpopulation (ipRGCs) expresses the photopigment melanopsin (Opn4) and project to brain areas controlling light-regulated activities (entrainment of rhythms, pupillary light reflexes (PLR), etc.). Blind chickens (*GUCY1**) lacking functional photoreceptor cells do express Opn4, exhibit PLR and synchronize their feeding rhythms to light after brain occlusion. Here, we investigated light and circadian-regulation of RGC activities and their potential roles in the non-visual circuit function. Chicken retinas from animals maintained in constant dark (DD), light (LL), or a 12:12 h LD cycle for 48 h,

were lyophilized and processed. Consensual PLR in dark-adapted GUCY1* chickens exposed to white or blue bright lights at different circadian times (CT) in DD, and feeding rhythms after synchronization to diverse LD cycles were assessed.

AA-NAT activity in RGCs displays a circadian rhythmicity with highest levels during day in DD, LL or the light phase of a LD cycle; however, a brief light pulse or dopamine administration (50 nmol/eye) had no effect on AA-NAT activity. The ipRGC regulated-photic entrainment of feeding rhythms was evaluated in GUCY1* birds after head occlusion to avoid extraocular photoreception; birds synchronized their feeding rhythms to LD cycles with L>12 lux. When released to LL, blind chickens became arrhythmic; however, after head occlusion, they free-ran with a 24.5 h period. To further characterize the ipRGC circuitry, we found that PLRs in GUCY1* birds were subject to a daily variation after white or blue light exposure with maximum constriction at CT6.

Results suggest that RGC oscillators and ipRGCs are part of a non-visual circuit in birds controlling melatonin synthesis locally, and PLRs with maximal responses at midday.

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IRB Status: International

Disclosures:

MARIO GUIDO, PHD: No financial relationships to disclose

0364

THE ROLE OF MELANOPSIN IN THE MAMMALIAN RETINAL CLOCK

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INSERM U846, STEM CELL AND BRAIN INSTITUTE

The mammalian retina contains an endogenous pacemaker that regulates retinal physiology and adjusts daily the temporal phase of the central circadian timing system with environmental time. This entrainment process involves rods, cones and intrinsically photosensitive retinal ganglion cells (ipRGCs). These ipRGCs express the photopigment melanopsin and has been shown to regulate a wide array of non-image forming visual processes such as the pupil light reflex and photoentrainment of circadian clocks. However, the role of melanopsin in retinal clock functions is still unclear.

Using a melanopsin knockout mouse, we found that the absence of melanopsin leads to a dysfunction of the clock mechanism mostly in the photoreceptors layer, characterized by changes in circadian clock gene expression and light-induction of *Per1* and *Per2* clock genes. Furthermore, removal of melanopsin prevented the light-dependent increase of tyrosine-hydroxylase mRNA and of dopamine. Since ipRGCs are known to provide a feedback signaling pathway for photic information to dopaminergic cells in the retina, these results suggest that melanopsin and its signaling through dopamine are involved in the functioning of the clockwork in the photoreceptors.

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IRB Status: None

Disclosures:

OURIA DKHISSI-BENYAHYA, PHD: No financial relationships to disclose

0365

MULTIPLE PATHWAYS FOR THE CIRCADIAN REGULATION OF THE RETINAL PHAGOCYTOSIS RECEPTOR MERTK

CELIA PARINOT, Jonathan Chatagnon, Emeline Nandrot

INSTITUT DE LA VISION

Clearance of aged photoreceptor outer segments (POS) by cells from the retinal pigment epithelium (RPE) is rhythmic and peaks 2 hours after light onset. Activity of the internalization receptor MerTK can be regulated through intra- and extracellular pathways. MerTK is phosphorylated at peak phagocytosis time via intracellular signaling pathways initiated by the α 5 β 1 integrin-MFG-E8 couple. Regulation via extracellular molecules proceeds through: (1) its 2 ligands Gas6 and Protein S, and (2) rhythmic cleavage of its extracellular domain and production of a soluble form (sMerTK). So far, the exact role of MerTK ligands *in vivo* is still unknown and the protease responsible for its cleavage has not been characterized. We explored the expression of MerTK's ligands and proteases at different times of the day using qPCR, immunoblotting, ELISA assays and immunofluorescence. *Gas6* gene expression in separated retina and RPE/choroid presents no extensive variation, whereas *Protein S* expression increases at phagocytic peak time. Protein levels for each ligand follow a similar profile compared to their respective gene expression. Among the protease candidates, HtrA1 and members of the ADAM's family of metalloproteases, ADAM9, 10 and 17 display a peak of gene and/or protein expression around peak phagocytosis time. However, only ADAM17 is located in the microvilli of RPE cells. Our *in vitro* data suggest that Gas6 inhibits POS phagocytosis while Protein S stimulates it, thus bearing opposite roles. Taken together, our present results show that Gas6 may downregulate MerTK activity at all times, whereas Protein S might potentiate its activation on time for the phagocytic peak. In addition, our *in vitro* digestion experiments show that ADAM17 cleaves MerTK and that it might require Gas6. Therefore, ADAM17 can be considered as a strong candidate for MerTK cleavage during daily POS phagocytosis *in vivo*.

IRB Status: None

Disclosures:

CELIA PARINOT, MSC: No financial relationships to disclose

CIRCADIAN REGULATION OF PHOTOPIC VISUAL FUNCTION: DIFFERENTIAL ROLES OF NPAS2 AND CLOCK

MICHAEL IUVONE, Christopher Hwang

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Retina contains circadian clocks that regulate gene expression and physiological functions, such as melatonin biosynthesis, rod-cone coupling, and photoreceptor disk shedding. In addition, retinal clocks regulate aspects of photopic (bright light) vision, including the sensitivity for detecting contrast and light-evoked electrical responses, as measured by electroretinographic (ERG) amplitudes. The positive loop of the circadian oscillator is driven by BMAL1/CLOCK or BMAL1/NPAS2 heterodimers acting on E-box enhancer elements. The current study was conducted to localize CLOCK and NPAS2 in the mouse retina and to assess the relative roles of CLOCK and NPAS2 in the daily rhythms of contrast sensitivity and photopic ERG. CLOCK is widely distributed in the retina as determined by immunohistochemistry, with highest expression in the inner nuclear layer (INL) and ganglion cell layer (GCL). In contrast, NPAS2 localization, as assessed by an NPAS2-lacZ reporter, is largely restricted to retinal ganglion cells. Photopic ERG b-wave amplitudes are higher during the subjective day than during the subjective night. This rhythm was abolished in *Clock*^{-/-} mice by selectively reducing daytime responses, but was not significantly affected in *Npas2*^{-/-} mice. The ability to detect contrast is also significantly higher during the subjective day than the subjective night. The amplitude of the circadian rhythm of contrast sensitivity was reduced but not abolished in *Npas2*^{-/-} mice, with reduction in daytime sensitivity. To investigate a possible molecular basis for this effect, the expression of a previously identified clock-controlled gene, *Adcy1*, was investigated in microdissected GCL, INL, and photoreceptors. *Adcy1* mRNA expression was rhythmic in photoreceptors and the GCL, but not in the INL. Consistent with the localization of NPAS2, the rhythm of *Adcy1* expression was abolished in the GCL but not in photoreceptors. Remarkably, the amplitude of the contrast sensitivity rhythm was reduced in *Adcy1*^{-/-} mice in a manner indistinguishable from that in *Npas2*^{-/-} mice, suggesting a causal link between NPAS2-regulated *Adcy1* expression and contrast sensitivity. Moreover, BMAL1/NPAS2 was shown to directly activate *Adcy1* promoter-luciferase constructs in an E-box dependent manner in transiently transfected NG108-15 cells. CLOCK and NPAS2 were co-localized in the GCL. Interestingly, no rhythm of contrast sensitivity was observed in *Clock*^{-/-} mice, in which *Npas2* and *Adcy1* rhythms were abolished in the GCL. Thus, the circadian regulation of contrast sensitivity involves CLOCK and NPAS2, while the rhythm of photopic ERG is mediated by CLOCK in an NPAS2-independent manner.

IRB Status: None

Disclosures:

MICHAEL IUVONE, PHD: No financial relationships to disclose

RC02 – Retinal TRP Channels and Visual Function

TRPV1 MODULATES VISUAL RESPONSES IN THE RETINA

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Non-selective cation transient receptor potential vanilloid-1 (TRPV1) channels were first located on peripheral terminals of nociceptive fibers (Moreira et al., 2011). Expression in mammalian retina has been documented using antibodies, but not verified with knockout (KO) or knockin (KI) mice. We evaluated expression in a *TrpV1KI* reporter mouse and recorded and compared extracellular spiking activity of WT and *TrpV1KO* RGCs. Responses were recorded from the optic nerve *in vivo* anesthetized mice preparation at light adapted levels. Stimuli characterized RGC receptive field (RF) center/surround organization and other RF response properties. Similar to WT, many *TrpV1KO* RGCs have RF center/surround organization and respond at either the onset (ON) or the offset (OFF) of light. The majority of *TrpV1KO* OFF RGCs responses are similar to WT; represented by two populations with either sustained or transient responses. The visually-evoked responses of *TrpV1KO* and WT ON RGCs differ significantly. WT ON-center RGCs are predominantly (97%) sustained, maintaining an excitatory response for ≥ 2 sec. In contrast, *TrpV1KO* ON-center RGCs are predominantly (70%) transient and only maintain an excitatory response for ≤ 0.4 s to the same stimulus. This transient excitation is followed by suppression of spiking below spontaneous activity that lasts during the remaining stimulus presentation. When we assessed the contribution of the receptive field surround using annular stimuli, we found that surround antagonism cannot be evoked in $\geq 50\%$ of *TrpV1KO* ON-center RGCs. Finally, a subset of *TrpV1KO* RGCs is very insensitive to visual stimulation and requires very high luminance levels to evoke a response (≥ 150 cd/m²). In TRPV1KI retina RGCs and other retinal cell classes are labeled. We conclude TRPV1 mediates an input in the ON pathway that shapes temporal responses of ON RGCs. This or other mechanisms contribute to differences in RF surround and light sensitivity.

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Disclosures:

MAUREEN MCCALL, PHD: No financial relationships to disclose

MECHANISMS GATING TRPM1 IN RETINAL ON-BIPOLAR CELLS

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The first synapse in the visual system is between the rod and cone photoreceptors and two classes of bipolar cells. One class of bipolar cells, the ON or depolarizing bipolar cells (DBC) require the G protein-coupled receptor, mGluR6, which signals via a G protein-coupled cascade to the transient receptor potential melastatin 1 (TRPM1) cation channel. When light intensity increases this cascade results in the depolarization of the DBCs, hence their name, initiating signaling through one of the parallel pathways. In addition to mGluR6, GPR179, another seven transmembrane protein is required for DBC function and recruits the regulators of G protein signaling (RGS) proteins, RGS7 and RGS11, to the dendritic tips of the DBCs. Here we utilize the *Gpr179^{nob5}* mouse to demonstrate that despite the absence of GPR179 and RGS7/RGS11, a small dark-adapted electroretinogram b-wave response remains and can be enhanced with long duration flashes. Consistent with the ERG, the mGluR6-mediated gating of TRPM1 can be evoked pharmacologically in *Gpr179^{nob5}* and *RGS7^{-/-}/RGS11^{-/-}* rod DBCs if strong stimulation conditions are used. In contrast, direct gating of TRPM1 by capsaicin in *RGS7^{-/-}/RGS11^{-/-}* and WT rod DBCs is similar, but severely compromised in *Gpr179^{nob5}* rod DBCs. Noise and standing current analyses indicate that the remaining channels in *Gpr179^{nob5}* and *RGS7^{-/-}/RGS11^{-/-}* rod DBCs have a very low open probability. We propose that GPR179 along with RGS7 and RGS11 controls the ability of the mGluR6 cascade to gate TRPM1. In addition to its role in localizing RGS7 and RGS11 to the dendritic tips, GPR179 via a direct interaction with the TRPM1 channel alters its ability to be gated directly.

IRB Status: None

Disclosures:

RONALD GREGG, PHD: No financial relationships to disclose

O369

ACTIVATION AND MODULATION OF THE TRPM1 CHANNEL IN ON-BIPOLAR CELLS

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In darkness, glutamate released from photoreceptors hyperpolarizes retinal ON bipolar cells by binding mGluR6, activating the heterotrimeric G-protein G_o , and closing TRPM1 channel. While the basic elements of this signaling cascade are known, the detailed interactions are far from understood. In this talk, we will address an unresolved issue: which G_o subunit, $G\alpha_o$ or $G\beta\gamma$ dimer, mediates the channel closure. We made whole-cell recordings from mouse rod bipolar cells perfused with Ames medium + strychnine and picrotoxin, and clamped at -60 mV. We dialyzed cascade modifiers through the recording pipette and recorded the TRPM1 channel behavior. The retina was either dark or light adapted and a light pulse (ON or OFF) was given every 35 seconds. Under light adaptation, dialyzing

GTP- γ -S quickly decreased the basal current and diminished the light OFF response, confirming that activated G protein cascade closes the channel. Dialyzing $G\alpha_o$ showed no change in either the basal current or the light OFF response. This suggests that $G\alpha_o$ does not close the channel. Interestingly, under dark adaptation, dialyzed $G\alpha_o$ increased the holding current significantly. Deactivating $G\alpha_o$ with GDP- β -S removed the effect, leaving the holding current as stable as in control cells. Dialyzing a constitutively active mutant of $G\alpha_o$ did not change the holding current. These results suggest that the channel opening by $G\alpha_o$ was mediated via inactive $G\alpha_o$. To test the effect of $G\beta\gamma$, we dialyzed under dark adaptation phosducin, a scavenger of $G\beta\gamma$. The basal current increased significantly and the light ON response increased slightly as well. A mutant phosducin that lost the ability to bind $G\beta\gamma$ did not change the current. This result is consistent with $G\beta\gamma$ closing the channel. Overall, our results suggest that inactive $G\alpha_o$ maintains the channel open, while free $G\beta\gamma$ closes the channel.

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O370

TRPV CHANNELS REGULATE NEURONAL AND GLIAL PHYSIOLOGY IN THE MAMMALIAN RETINA

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Osmotic fluxes and mechanical displacement are inherent in eye growth and retinal function, yet the role of osmotic swelling/shrinking and tensile/compressive stretch in visual signaling remains unclear. Here we show that retinal neurons and glia use ion channels, TRPV1 and TRPV4 (transient receptor potential vanilloid isoforms 1 & 4) to respond to osmotic and mechanical stretch. Exposure to hypotonic/hypertonic saline and biaxial cyclic strain elicited inward cation currents and calcium elevations in mouse retinal ganglion cells (RGCs) and Mueller glial cells but not photoreceptors, bipolar or amacrine cells. These signals were sensitive to TRPV channel blockers and were reduced in cells from retinas with ablated TRPV channels. Neuronal and glial TRPV4 signals showed marked differences in the onset kinetics and duration, together with differential sensitivity to the modulators of the arachidonic acid signaling pathway. Activation of TRPV4 increased RGC excitability and, if sustained, resulted in excitotoxicity. Glial TRPV4 activation was associated with MAPK signaling and, if sustained, resulted in the induction of reactive gliosis. Thus, we have identified a mechanism through which mechanical stimuli differentially regulate neuronal and glial activation within the retina.

IRB Status: None

Disclosures:

DAVID KRIZAJ, PHD: Equity Owner relationship with Asha Vision, LLC

RC03 – Retinal Calcium-Binding Proteins: Structure, Function, and Role in Disease

O371

GUANYLYL CYCLASE-ACTIVATING PROTEINS IN SIGNAL TRANSDUCTION AND RETINAL DISEASES: DO THEY COMPETE FOR THE SAME TARGET ENZYME OR ACT SYNERGISTICALLY?

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Calcium-sensor proteins GCAP1 and GCAP2 activate retinal membrane guanylyl cyclases (RetGC1 and RetGC2) when Ca^{2+} concentrations in mammalian photoreceptors fall in the light and through that activation accelerate the recovery. Multiple mutations in GCAP1 (coded in humans by *GUCA1A* gene) and RetGC1 (human gene *GUCY2D*) shift Ca^{2+} sensitivity of RetGC and, by increasing the intracellular cGMP and Ca^{2+} levels, trigger severe retinal degenerations—such as CORD or dominant cone degeneration. Both GCAP isoforms can activate RetGC1 and RetGC2 *in vitro*. However, GCAP1 *in vivo* preferentially targets RetGC1, while GCAP2 activates both RetGC1 and RetGC2. Since the two GCAPs can both target the same RetGC1 isozyme in living rods, do they compete with each other over RetGC1 or act synergistically? We find that at near-saturating concentrations, GCAP1 and GCAP2 do not produce any additive effect on RetGC1 activation. As yet another evidence to support mutually exclusive activation of RetGC1 by GCAP1 and GCAP2, a GCAP1 mutant, which strongly binds RetGC1 but fails to activate it, effectively competes with both GCAP1 and GCAP2 and suppresses cyclase stimulation by both GCAP isoforms in a similar fashion. We therefore reason that, regardless of the question about their binding sites on RetGC1 being overlapping or non-overlapping with each other, only one of the two GCAPs can form an active complex with the same RetGC1 subunit at a time.

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Disclosures:

ALEXANDER DIZHOOR, PHD: No financial relationships to disclose

O372

STRUCTURAL DIVERSITY OF NEURONAL CALCIUM SENSOR PROTEINS AND INSIGHTS FOR ACTIVATION OF RETINAL GUANYLYL CYCLASE BY GCAP1

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Neuronal calcium sensor (NCS) proteins, a sub-branch of the calmodulin superfamily, are expressed in the brain and retina where they transduce calcium signals and are genetically linked to degenerative diseases. The amino acid sequences of NCS proteins are highly conserved but their physiological functions are quite different. Retinal recoverin controls Ca^{2+} -dependent inactivation of light-excited rhodopsin during phototransduction, guanylyl

cyclase-activating proteins (GCAP1 and GCAP2) promote Ca^{2+} -dependent activation of retinal guanylyl cyclases, and neuronal frequenin (NCS-1) modulates synaptic activity and neuronal secretion. In my seminar, I will discuss the molecular structures of myristoylated forms of NCS-1, recoverin, and GCAP1 that all look very different, suggesting that the attached myristoyl group helps to refold these highly homologous proteins into different three-dimensional folds. Ca^{2+} -binding to both recoverin and NCS-1 cause large protein conformational changes that ejects the covalently attached myristoyl group into the solvent exterior and promotes membrane targeting (Ca^{2+} -myristoyl switch). The GCAP proteins undergo much smaller Ca^{2+} -induced conformational changes and do not possess a Ca^{2+} -myristoyl switch. Recent structures of GCAP1 in both its activator and Ca^{2+} -bound inhibitory states will be discussed to understand structural determinants that control Ca^{2+} -dependent activation of retinal guanylyl cyclases.

IRB Status: None

Disclosures:

JAMES AMES, PHD: No financial relationships to disclose

O373

IDENTIFICATION OF THE INTERFACE FOR THE TARGET ENZYME ACTIVATION IN GCAP1

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Guanylyl cyclase activating proteins (GCAPs) play a critical role in regulation of retinal guanylyl cyclase (RetGC) in vertebrate photoreceptors, but GCAP interaction with its target enzyme remains obscure. In the present study, we mapped GCAP1 residues comprising the RetGC1 binding site by mutagenizing the entire surface of GCAP1 and testing the ability of each mutant to bind RetGC1 in a cell-based assay and to activate it *in vitro*. We found that the interface for the cyclase is located on one side of the GCAP1 molecule and is fairly compact. Mutations that most strongly affected activation of RetGC1 localized to a distinct patch formed by the surface of non-metal binding EF-hand 1, the loop and the exiting helix of EF-hand 2, and the entering helix of EF-hand 3. These mutations also prevented binding of GCAP1 to RetGC1. In contrast to these three EF-hands, mutations in the EF-4 and helices 10 and 11 had little effect on cyclase activation by GCAP1. In spite of being an essential EF-hand for switching the cyclase on and off by the conformational changes induced by Ca^{2+} binding, the surface of the EF-hand 4 contributes rather little to the binding of RetGC1. We also identified a few residues near the binding patch (Met26, Lys85 and Trp94) which were critically important for activation of the cyclase but not for GCAP1 binding to RetGC1. Mutations of these residues strongly affected activation of RetGC1, yet did not prevent binding of GCAP1 to RetGC1. We propose the two-step mechanism, in which the first step involves primary binding to RetGC followed by a second step that induces secondary contacts important for cyclase activation by GCAP1.

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Disclosures:

IGOR PESHENKO, PHD: No financial relationships to disclose

DIVERSITY OF NEURONAL CALCIUM SENSOR PROTEINS IN ZEBRAFISH RODS AND CONES

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UNIVERSITY OF ZURICH, MOLECULAR LIFE SCIENCES²

Zebrafish has recently become a favorite model organism in vision research and photoreceptor specific proteins are often expressed in a larger variety than in the mammalian retina. Six different isoforms of guanylate cyclase-activating proteins (zGCAPs) that belong to the family of neuronal calcium sensor (NCS) proteins were identified in zebrafish rods and cones. They regulate three different membrane-bound guanylate cyclases thereby exhibiting differences in Ca^{2+} -binding, sensing and activity modulation. By sensing incremental changes of cytoplasmic Ca^{2+} -concentration in rod and cone cells they control the activity of their target guanylate cyclases in a Ca^{2+} -relay mode fashion. Recoverin, another NCS protein first described in the mammalian retina, is also found in the zebrafish genome. Four individual members of the recovering gene family, *rcv1a*, *rcv1b*, *rcv2a* and *rcv2b* are expressed in photoreceptors. Recombinant proteins are myristoylated and all seem to undergo a Ca^{2+} -myristoyl switch, but they differ in their Ca^{2+} -sensing properties. So far, only recoverin 1a and 2a seem to interact with the N-terminus of opsin kinase GRK7a. In summary, NCS proteins in zebrafish rods and cones constitute an intricate network of Ca^{2+} -sensing proteins that operate in extending the dynamic range of target regulation in response to fluctuating intracellular Ca^{2+} .

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EML1 (CNG-MODULIN) MODULATES CONE CGMP-GATED CHANNEL AND CONTROLS LIGHT SENSITIVITY IN FISH RETINAL CONE PHOTORECEPTORS

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CNG-modulin is a Ca^{2+} -binding protein expressed in retinal cone photoreceptors in striped bass. The protein mediates a Ca^{2+} -dependent modulation of ligand sensitivity of cGMP-gated (CNG) ion channels. Through comparative genomic analysis we identified the orthologue gene of CNG-modulin in zebrafish, *eml1*. We investigated the functional role of CNG-modulin in phototransduction *in vivo* in morpholino-mediated EML1 knockdown in zebrafish. We compared the photoresponses of wild-type cones with those of cones that do not express

the EML1 protein. In the absence of EML1, dark adapted cones are ~5.3-fold more light-sensitive than wild-type cones. Previous qualitative studies in several non-mammalian species have shown that immediately after the onset of continuous illumination cones are less light-sensitive than in darkness, but sensitivity then recovers over the following 15-20 seconds. In wild type zebrafish larvae, the response to a flash presented about 1 second after background onset is smaller in amplitude than that generated by the same flash in darkness. However, despite the continuing presence of background light, the response to the same flash becomes progressively larger as the interval between background onset and flash presentation increases. This amplitude recovery has an exponential time course with 2-8 sec time constant, depending on the intensities of both background and test flash. In the morphant larvae, the response to a flash delivered 1 sec after background onset is also smaller than in the dark. However, in the morphant larvae the light-adapted flash response does not change in amplitude over time, that is, sensitivity recovery does not occur in the absence of EML1.

IRB Status: Approved

Disclosures:

TATIANA REBRIK, PHD: No financial relationships to disclose

RC04 – Retinal Angiogenesis

THE ROLE OF MYELOID-DERIVED CELLS IN INFLAMMATION-MEDIATED RETINAL ANGIOGENESIS

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Sight-threatening retinal diseases, including age-related macular degeneration (AMD), diabetic retinopathy and uveoretinitis have pathological components of chronic inflammation and angiogenesis. We have shown that chronic inflammation in the experimental autoimmune uveoretinitis (EAU) induces intraretinal angiogenesis. Myeloid-derived cells, in particular macrophages, are critically involved in inflammation-mediated angiogenesis. Macrophages can be differentiated from circulating monocytes that express either $\text{CCR2}^+\text{CD62L}^{\text{hi}}$ (classical) or CX3CR1^+ (non-classical). The angiogenic potential of different subsets of monocytes may differ. In experimental mice, chronic EAU-induced retinal angiogenesis and laser-induced choroidal neovascularisation (CNV) were significantly reduced in mice with CCR2 or CCL2 deficiency, but not CX3CR1 deficiency. Circulating monocytes from neovascular AMD (nAMD) patients express higher levels of CX3CR1 , MHC-II, HLA-DR and the Signal Transducer and Activator of Transcription 3 (STAT3), suggesting that uncontrolled monocyte activation. Depletion of the Suppressor Of Cytokine Signaling 3 (SOCS3) in myeloid cells results in STAT3 over-expression and constitutive cell activation. EAU-induced retinal angiogenesis and laser-induced CNV were significantly increased in myeloid-specific SOCS3 deficient mice. Our results suggest that the CCR2^+ myeloid-derived cells play an important role in inflammation-mediated retinal angiogenesis,

and SOCS3 can negative regulate the angiogenic potential of myeloid-derived cells under inflammatory conditions.

IRB Status: Approved

Disclosures:

HEPING XU, MD, PHD: No financial relationships to disclose

O377

NUCLEAR RECEPTOR RORALPHA CONTROL OF PATHOLOGIC RETINAL NEOVASCULARIZATION

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Pathologic neovascularization in the eye is a leading cause of blindness, associated with dysregulated lipid metabolism and inflammation. RORalpha is a lipid-sensing nuclear receptor that controls inflammation. Mutations in RORalpha were associated with increased risk of developing neovascular age-related macular degeneration, suggesting an important role of RORalpha in regulating pathologic neovascular growth in the eye. Yet studies on RORalpha control of ocular vascular growth is lacking. Using a mouse model of oxygen-induced proliferative retinopathy (OIR), we found that expression of RORalpha is significantly upregulated in the proliferative phase of retinopathy, and RORalpha deficient mice show strong suppression of pathologic neovascularization in OIR. This RORalpha mediated vascular effect is associated with altered inflammatory cytokine expression and macrophage polarization. We found RORalpha directly controls transcription of *Socs3* (suppressor of cytokine signaling 3), a critical regulator of macrophage polarization, through interaction with the promoter region of *Socs3*. Blocking *Socs3* in macrophages abolishes RORalpha regulation of inflammatory cytokine production. Together our data suggest a novel role of RORalpha in controlling pathologic vascular growth in proliferative retinopathy, through modulating macrophage polarization and inflammation. Targeting RORalpha may represent a new strategy to develop therapeutics for treating or preventing pathologic vessel proliferation in eye diseases.

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IRB Status: None

Disclosures:

JING CHEN, PHD: No financial relationships to disclose

O378

LRG1: A NEW THERAPEUTIC TARGET FOR THE TREATMENT OF RETINAL NEOVASCULAR COMPLICATIONS?

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We have recently reported the discovery of a novel regulator of pathological angiogenesis named leucine-rich alpha-2-glycoprotein 1 (LRG1) (Wang et al., *Nature*, 2013 499:306-11). In this study we showed that during retinal and choroidal neovascularization LRG1 is significantly up-regulated and that it mediates its angiogenic activity via modulation of endothelial TGFβ signaling. In addition, LRG1 blockade using a polyclonal antibody or *Lrg1* gene knockout, leads to smaller neovascular lesion size in laser-induced choroidal neovascularization (CNV) and oxygen-induced retinopathy (OIR).

Here we report the development of a LRG1 function-blocking monoclonal antibody that has the potential to be used instead of, or in combination with, VEGF blockade in the treatment of retinal vascular disease. More than 100 antibodies were generated and screened initially using surface plasmon resonance to eliminate those with an affinity <1nM. Those that passed the first screen were then tested for blocking activity using the co-culture model of angiogenesis in which human umbilical vein endothelial cells form vessels on a monolayer of fibroblasts. The most effective antibodies from this screen were then evaluated for their ability to block lesion formation in both the mouse and rat models of laser-induced CNV.

Our work has led to the identification of a lead LRG1 function-blocking monoclonal antibody that is suitable for onward humanization and assessment in clinical trials. We propose that this antibody may be useful in the treatment of conditions such as proliferative diabetic retinopathy and neovascular age-related macular degeneration, in patients that are either refractory or poorly responsive to VEGF blockade, or in combination with VEGF blockers to achieve improved clinical outcomes.

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IRB Status: None

Disclosures:

JOHN GREENWOOD, PHD: No financial relationships to disclose

O379

NETRIN-4 DOWNREGULATION MODULATES PATHOLOGIC OXYGEN-DRIVEN NEOVASCULARIZATION

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The pathological formation of blood vessels is mostly accompanied by degeneration of the neuronal tissue in the retina. Netrins are a laminin-related family of matrix-binding secreted proteins, which were first identified as long distance guidance signals in normal and pathological neuroangiogenesis. Surprisingly, netrin-4, the newest member of the netrin family, appeared recently to display anti-angiogenic properties.

Methods: Wild-type and netrin-4 knockout (*Ntn4*^{-/-}) mice exposed to hyperoxia from P7 to P12 (75% O₂) and examined

the days P12, 17, 21 and 28. Laser-induced inflammatory chorioidal neovascularization served as a control model. Immuno-histochemical stainings from retinal flatmounts and paraffin sections were analyzed by image-processing and subsequent statistical evaluation. mRNA and protein expression were assessed at the respective time points. Retinal function was monitored by in vivo electroretinography (ERG).

Results: In wild-type mice netrin-4 localized to the basal membrane of the large blood vessels in the inner retina. Under control conditions, no differences in the retinal structure were found in Ntn-4^{-/-} mice and their wild-type littermates and the VEGF expression rates were comparable in both strains. However, compared to wild-type mice, Ntn-4^{-/-} mice displayed after relative hypoxia larger avascular areas at P12 which recovered much faster leading to significant more complete re-vascularization at P17. Along with this, the corresponding upregulation in VEGF expression occurred in the Ntn-4^{-/-} mice already at P14 whereas in wild-type mice at P17. Furthermore, the netrin-4 expression was oxygen-sensitive: under control conditions the netrin-4 expression increased steadily until day P21 whereas under relative hypoxia netrin-4 expression decreased until day P17 and recovered to normal levels at day P21. The structural recovery in Ntn-4^{-/-} mice could also be detected in functional ERG analysis indicated by faster recovery of a- and b-wave amplitudes at day P21. The expression of the putative netrin-4 receptors neogenin, Unc5H2 and DCC was neither changed in Ntn-4^{-/-} conditions nor after relative hypoxia. There was no difference between Ntn-4^{-/-} and their respective controls in laser-induced neovascularization.

Discussion: Our results indicate a role for netrin-4 mainly in hypoxia-induced angiogenesis. Netrin-4 is enriched in the basement membrane of large blood vessels in normoxia, however down-regulated after hypoxia. The hypoxia-dependent decrease of netrin-4 expression in the basal membrane seems to be a signal for increased VEGF production e.g. by Mueller cells. In summary, netrin-4 stabilizes the blood vessel architecture under normoxic conditions but its down-regulation in response to hypoxia represents the prerequisite to ignite neovascularisation.

IRB Status: International

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ANTONIA JOUSSEN, MD, PHD: No financial relationships to disclose

O380

AND THEN THERE WERE MILLIONS: NEW ANTIBODY THERAPY FOR ANGIOGENESIS

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Bevacizumab (Avastin™), a humanized IgG1 monoclonal antibody (mAb), is widely used in neovascular age-related

macular degeneration (AMD) and in numerous other ocular and systemic conditions such as cancers. Surprisingly, although bevacizumab does not recognize murine Vegfa, it was reported to inhibit angiogenesis in the mouse.

We tested bevacizumab in three mouse models of angiogenesis and showed that it inhibits neovascularization not via Vegfa blockade but rather through Fcγ Receptor I (FcγRI) via its Fc region, causing impaired macrophage migration. Other approved humanized or human IgG1 antibodies (adalimumab, alemtuzumab, ofatumumab, omalizumab, palivizumab, tocilizumab) that lack mouse targets, as well as intravenous immunoglobulin (IVIg), also inhibited angiogenesis in mice via FcγRI. These antibodies also suppressed angiogenesis in FcγR humanized mice, which express the human, but not the mouse, Fcγ receptors. FcγR1 ablation, Fc enzymatic cleavage, IgG-Fc peptide inhibitor, or removal of sugar residues required for IgGs to engage FcγRI, abolished this class effect. Furthermore, the Fc region potentiated the anti-angiogenic activity of bevacizumab in humanized VEGFA mice.

We have identified a novel target-independent, FcγRI-dependent anti-angiogenic class effect of hIgG1 antibodies and IVIg, which are used in millions of patients worldwide. Our findings offer new, and potentially inexpensive, therapeutic opportunities for repurposing existing, approved drugs as angioinhibitors for blinding diseases such as neovascular AMD, proliferative diabetic retinopathy, and a multitude of systemic disorders. These data also strike a cautionary note for the need for greater vigilance in the use of hIgG1 and IVIg therapies not intended to suppress angiogenesis.

IRB Status: Approved

Disclosures:

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O381

DARK REARING (DR) AS A MEANS OF MIMICKING PHYSIOLOGICAL HYPOXIA: A NON-INVASIVE INTERVENTION FOR RETINOPATHY OF PREMATURITY (ROP)

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The initiating event in ROP takes place when premature infants are placed in high oxygen to protect the brain from damage. However, because the choroid has limited autoregulatory ability, the inner retina becomes flooded with oxygen. Higher-than-normal oxygen down-regulates VEGF, reducing rate and density of retinal vasculature formation, such that when infants no longer require oxygen supplementation, retinal vasculature formation is mismatched to neuronal metabolic demand, resulting in tissue hypoxia and pathological vaso-pro-

liferation. We hypothesized neonates in hyperoxia be subject to total darkness, creating a “metabolic sump”, maintaining physiological VEGF expression.

To test this hypothesis, SD rats were raised in: room air+DR from P0-P30; hyperoxia (58%/72% O₂ from P0-4, return to room air+normal light) with and without DR and assessed at P7; and under DR/normal light in the Penn model of ROP. Retinae were examined for vascular density, avascular/neovascular area, pericyte/astrocyte ensheathment and ultrastructural change via TEM. Flash electroretinograms (fERG) were recorded in long-term DR rats (P30/60/90). Retinal oxygen profiles were modeled for P14 at 60 and 75% inspired oxygen in light and DR.

From P7-11, room air+DR rats had higher vascular density compared to controls (VDI=43±1.0vs.38±1.1 p<0.05). DR protects vessels from oxygen-induced vaso-obliteration (20.64% ± 4.85 v 39.02% ± 4.03 p<0.05) and reduces neovascularization (4.07% ± 0.85 v 15.79% ± 1.26 p<0.05) in the Penn model of ROP. DR is vasculoprotective in the Penn model and long-term DR has no detrimental effects on retinal cell function and morphology, as evidenced by fERG and TEM. Oxygen consumption modeling at P14 showed that at ~58% inspired oxygen, inner retina is hypoxic, maintaining VEGF expression in darkness, but not in light.

DR can preclude the initiation of ROP, offering a non-invasive intervention. This is timely, given enthusiasm for anti-VEGF therapy in ROP, where possible systemic toxicity has not been fully investigated.

IRB Status: International

Disclosures:

TAILOI CHAN-LING, PHD: No financial relationships to disclose

RC05 – Photoreceptor Cell Biology I

O382

AXONEME ORGANIZATION AND IFT IN ZEBRAFISH AND MAMMALIAN PHOTORECEPTORS

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Vertebrate photoreceptor outer segments (OS) arise from cilia and the cytoskeletal components of the cilium called the axoneme are critical to their structural organization. As a consequence we have focused on axoneme structure and on the formation of the axoneme through intraflagellar transport (IFT). We have previously proposed a model, similar to that in *C. elegans* sensory cilia, in which both members of kinesin 2 family of motors, the heterotrimeric kinesin 2 and homodimeric Kif17, function within the OS. We have now produced a new mutant allele of Kif17 using TALEN mediated genome editing that carries an 11 bp deletion in exon 1. This mutation leads to defects in the organization of the outer retina, particularly in the cone tiers, and loss of cones, further supporting an important role for Kif17 in photoreceptor maintenance. Rods in both zebrafish and mice have a transition zone (often called the connecting cilium) and the axoneme can be traced only a short distance into the

OS. In contrast, cone axonemes generally extend the full length of the OS. In zebrafish we have found that cone axonemes frequently extend distally beyond the OS tip and that Kif17-GFP accumulates at the tip. To further study cone axoneme organization we have produced stable lines of zebrafish expressing alpha-tubulin, KIF17 and IFT proteins, all labeled with a fluorescent protein (EOS, GFP, mCherry) and driven by a cone specific promoter. Confocal analysis of fluorescent proteins combined with electron microscopic analysis of tannic acid stained retina, which delineates microtubule protofilaments, has enabled us to validate the existence of distal extensions that were originally identified in isolated cells. Since distal extensions extend deep into the RPE apical processes we suggest that they function in axial alignment of cones, which are arranged in tiers in zebrafish.

IRB Status: None

Disclosures:

JOSEPH BESHARSE, PHD: No financial relationships to disclose

O383

RAPID PHOTORECEPTOR DEGENERATION OCCURS IN ZEBRAFISH *ARL13B* MUTANTS FOLLOWING SUPPRESSION OF PLANAR CELL POLARITY SIGNALING

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CLEVELAND CLINIC

Mutations in *Arl13b* are linked to Joubert Syndrome, a ciliopathy characterized by a distinctive midbrain-hindbrain malformation, kidney cysts, and varying degrees of retinal degeneration. *Arl13b* localizes to cilia but its function(s) are poorly understood. Mice and zebrafish with *Arl13b* mutations exhibit cilia defects but retinal phenotypes have not been explored. As genetic interactions between mutant alleles of cilia loci can affect pathological expression, *Arl13b* may interact with other loci to promote retinal degeneration. We hypothesized that *Arl13b* and components of the Planar Cell Polarity pathway function together to enhance the *Arl13b* phenotypic spectrum within photoreceptors. Zebrafish *Arl13b* mutant photoreceptors did not exhibit overt signs of retinal degeneration by electron microscopy or immunohistochemistry at 5 days post fertilization (dpf). Using genetic mosaic analysis, *Arl13b* mutant photoreceptors began degenerating by 14 dpf and the majority were completely lost by 30 dpf following transplantation into wild-type host animals. Co-injection of sub-phenotypic doses of morpholinos against *Arl13b* and PCP genes, such as *vangl2* and *fuzzy*, or the ciliopathy gene *bbs8* resulted in the loss of photoreceptor outer segments and cilia. In double morphants, basal bodies were abnormally positioned and many failed to dock at the apical surface, indicating that *Arl13b* functionally interacts with the PCP pathway to control cilia docking and outer segment morphogenesis. Our results suggest that loss of *Arl13b* results in a slow retinal degeneration but genetic interactions between *Arl13b* and *bbs8* or components of the PCP pathway suggest that modifying alleles of PCP genes may enhance photoreceptor degeneration in ciliopathies.

IRB Status: Approved

Disclosures:

BRIAN PERKINS, PhD: No financial relationships to disclose

O384**OUTER SEGMENT PROTEIN DELIVERY RELIES ON A VARIETY OF TARGETING MOTIFS****VADIM ARSHAVSKY¹, Jillian Pearing¹, Raquel Salinas¹, Eric Lieu¹, Sheila Baker²***DUKE UNIVERSITY¹; UNIVERSITY OF IOWA²*

Maintaining normal functioning and supporting healthy status of photoreceptor cells is critically dependent on targeted delivery of signaling and structural proteins to their light-sensitive outer segment compartment. Despite a wealth of information regarding rod outer segment targeting of rhodopsin, little is known about the mechanisms responsible for trafficking other outer segment-resident proteins. One strategy to approach this problem is to identify the information encoded within these proteins' sequences that is critical for outer segment delivery. Our laboratory investigates the targeting of three outer segment proteins: peripherin, RG59 anchor protein (R9AP) and the beta-subunit of the cGMP-gated channel (CNGβ1). To this end, we have found that the targeting sequence of peripherin is encoded within a stretch of ten C-terminal amino acids, with only a single valine residue being indispensable. The targeting sequence of R9AP was found to be confined within its SNARE homology domain, an important observation revising our previous conclusion that outer segment trafficking of this protein is piggybacked on that of rhodopsin. Finally, we showed that CNGβ1 does not rely on a C-terminal RVXP targeting sequence (as reported for olfactory CNGβ1b), but instead contains a distinct targeting domain present in its N-terminus. Interestingly, all of these proteins are delivered to the outer segment independently of rhodopsin, as evident from their reliable targeting to the rudimentary outer segments of rhodopsin knockout mice. What is perhaps most intriguing about these findings is the lack of any overt sequence homology across these proteins' targeting domains. This either suggests multiplicity of outer segment targeting mechanisms or indicates that the recognition of these domains occurs at the higher, tertiary structure level.

IRB Status: None

Disclosures:

VADIM ARSHAVSKY, PHD: No financial relationships to disclose

O385**STRUCTURES AND PATHWAYS FOR MEMBRANE TARGETING IN RODS****THEODORE WENSEL, Feng He, Melina Agosto, Zhixian Zhang, Michael Schmid***BAYLOR COLLEGE OF MEDICINE, BIOCHEMISTRY AND MOLECULAR BIOLOGY*

Rod photoreceptor cells in the vertebrate retina experience an enormous volume of membrane traffic in order to maintain a flow of material needed for phototransduction into the modified primary cilium known as the rod outer segment (ROS), which loses close to ten percent of its mass every day due to phagocytosis of distal disks by adjacent retinal pigmented epithelium

cells. Our laboratory uses a variety of techniques to uncover the structures and biochemical mechanisms necessary to maintain this complex membrane traffic and sorting, and to alter it in response to light exposure and other environmental stresses. We combine biochemical analysis of phosphoinositides and other lipids, cryo-electron tomography and conventional electron microscopy, as well as fluorescence microscopy, with genetically engineered mouse models and plasmid electroporation into rods of wildtype mice, to determine the structures associated with normal primary cilium function and traffic, the alterations in lipid composition and membrane structure induced by light, and how these are disrupted by disease-causing genetic deficiencies. Advances in structural analysis by sub-tomogram averaging, and phenotypic characterization of mouse models of ciliopathies and mice with cell-type specific ablation of the class III phosphoinositide 3-kinase, Vps34 will be presented.

IRB Status: Verified

Disclosures:

THEODORE WENSEL, PHD: No financial relationships to disclose

O386**RHODOPSIN TRAFFICKING AND MORPHOGENESIS OF OUTER SEGMENT DISK MEMBRANES****DAVID WILLIAMS¹, Stefanie Volland¹, Vanda Lopes¹, Julian Esteve¹, Steven Fisher²***UCLA, OPHTHALMOLOGY¹; UNIVERSITY OF CALIFORNIA, SANTA BARBARA, NEUROSCIENCE RESEARCH INSTITUTE²*

In the anabolic phase of outer segment disk membrane turnover, opsin and other membrane proteins move from the proximal inner segment to the cilium, then along the cilium to where the disk membranes are formed. Different hypotheses have now been proposed concerning the route opsin takes, how it is transported along the cilium, and how the disk membranes form. Most of the data underlying these hypotheses have come from traditional microscopy. We are now testing these hypotheses, using live cell imaging experiments of opsin delivery, and EM tomography to understand the architecture of disk membrane morphogenesis. Experiments, including FRAP analysis of RHO-GFP in the connecting cilium of mouse retinal explants, indicates that RHO is routed via the plasma membrane, and requires heterotrimeric kinesin-2 for normal ciliary transport, thus supporting transport of RHO along the ciliary plasma membrane, rather than in vesicles through the core of the cilium. EM tomography of rods from several species indicates that past EM studies have underestimated the 3-D complexity of the nascent outer segment disk membranes. In particular, tomograms, with z-axes of at least 250 nm, demonstrate that nascent disks can appear to be completely internal in some profiles, but then contain openings to the extracellular space at different positions along the z axis. These observations support the "open disk" hypothesis, but also demonstrate irregularities in the organization of the openings.

IRB Status: None

Disclosures:

DAVID WILLIAMS, PHD: No financial relationships to disclose

VISUALIZATION OF PROTEIN TRAFFICKING AND OUTER SEGMENT MORPHOGENESIS IN INTACT *XENOPUS LAEVIS* PHOTORECEPTOR CELLS

YOSHIKAZU IMANISHI

CASE WESTERN RESERVE UNIVERSITY

Rhodopsin, peripherin/rds, and cGMP gated channel are essential for the vertebrate vision. My laboratory studied the trafficking processes of these proteins by two novel techniques. In these techniques, proteins of interest were fused to a fluorescent protein Dendra2 and expressed in *Xenopus laevis* rod photoreceptors. One technique depends on quantitative microscopy which allows us to determine the outer segment (OS) concentrations and the degree of mislocalization. Another technique depends on photoconversion of Dendra2 which causes a shift of emission peak from green to red. This color shift allows us to discriminate between old and newly synthesized fusion proteins. By using this photoactivation technique, we studied the dynamic events associated with the renewal of membrane proteins.

The study of rhodopsin C-terminal region resulted in a discovery of a new "mis-trafficking signal," which confers toxicity to rhodopsin and causes mislocalization. This mislocalization signal is activated in the absence of the VXPX cilia targeting motif. We found that the VXPX motif has two roles: (1) It neutralizes the mislocalization signal. (2) It enhances ciliary targeting at least several folds, increasing the trafficking rate of rhodopsin carrier vesicles. Photoconversion technique revealed that, in the absence of the VXPX motif, mislocalized rhodopsin is eliminated from the cells through secretion of vesicles into the extracellular environment. Thus, this study clarified the roles of trafficking signals in rhodopsin trafficking, mis-trafficking, and renewal.

To understand the mechanism of OS morphogenesis, the photoconversion technique was applied to compare the OS trafficking of rhodopsin, peripherin/rds and cGMP-gated channel. By spatiotemporally tracking these three proteins, we were able to comprehensively study the morphogenesis process of three distinct domains of the OS: disk lamellar region, disk rim region, and OS plasma membrane. Using these novel, but simple methods, new insights are being gained into long standing questions of photoreceptor outer segment morphogenesis.

IRB Status: None

Disclosures:

YOSHIKAZU IMANISHI, PHD: No financial relationships to disclose

RC06 – Role of Mononuclear Phagocytes in Age-Related Macular Degeneration

PHENOTYPE AND FUNCTION OF PERIPHERAL BLOOD MONOCYTE FROM AMD PATIENTS

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Inflammation has been implicated in the pathogenesis of age related macular degeneration (AMD). While the complement system has a major role in that context, macrophages were also associated with AMD. To gain additional insight into monocyte and macrophage involvement in the disease, we have evaluated the phenotype and the function of these cells in AMD patients and controls. To that end, we have tested the expression of chemokine receptors on mononuclear cells and have assessed global gene expression patterns in monocytes from AMD patients and controls. We then differentiated the monocytes to macrophages and polarized them to the M1 and M2 phenotypes. Cells were tested for protein and gene expression profiles and for their contribution to the development of laser induced choroidal neovascularization (CNV) in rats. These studies suggested that the CD14+CD16+ monocyte subgroup from AMD patients show up-regulation of the CCR2 and CCR1 receptors. Furthermore, the entire monocyte population from AMD patient show a pro-inflammatory gene expression signal compared with the age-matched controls. Macrophages matured from AMD monocytes also show a pro-inflammatory signature that is enhanced in the M1 phenotype, while a pro-angiogenic signature is present in the M1 and M2 phenotypes compared with resting macrophages (M0). Accordingly, both the M1 and M2 phenotype demonstrated pro-angiogenic effect in the rat model of laser-induced CNV. Combined, these data implicated monocytes from the systemic circulation in the pathogenesis of AMD, and suggests that once matured to macrophages these cells may have a pro-angiogenic function in the context of CNV. Further research is required to assess if monocyte and macrophages may serve as therapeutic target in AMD.

IRB Status: Approved

Disclosures:

ITAY CHOWERS, MD: Consultant relationship with Novartis, Bayer, Allergan, and Lycored

MONONUCLEAR PHAGOCYTE SURVIVAL IN THE SUBRETINAL SPACE

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INSTITUT DE LA VISION¹; LABORATOIRE IMMUNITÉ ET INFECTION²; MIRCEN³

Age related macular degeneration (AMD) is associated with a breakdown of the subretinal immunosuppressive environment and infiltration of mononuclear phagocytes (MPs). The molecular mechanisms that lead to this alteration are unknown. We show that APOE expression is increased in *Cx3cr1*-deficient mice, which accumulate subretinal MPs, and in mice expressing the AMD-associated *APOe2* allele. We demonstrate that increased APOE secretion from MPs leads to prolonged subretinal MP survival and accumulation in *Cx3cr1*-deficient and *APOe2* mice. We show that subretinal MP accumulation in *APOe2* mice is associated with photoreceptor degeneration and excessive experimentally induced choroidal neovascularization, major hallmarks of late AMD. Our results highlight the inflammatory role of APOE in the immunosuppressive subretinal space and provide rationale for the increased AMD-risk of *APOe2* carriers.

IRB Status: Approved

Disclosures:

FLORIAN SENNLAUB, MD, PHD: No financial relationships to disclose

O390

NOVEL MARKERS AND THERAPY TARGETS FOR REACTIVE RETINAL MICROGLIA

THOMAS LANGMANN

UNIVERSITY OF COLOGNE

Reactivity of retinal microglial cells is a common hallmark of inherited retinal degenerations and age-related macular degeneration. Using various genetic mouse models including retinoschisin-deficient mice and light-damage as well as laser-damage protocols, we could show that the translocator protein 18 kDa (TSPO) is strongly induced in reactive retinal microglia. Moreover, the specific TSPO-ligand XBD173 was able in vitro and ex vivo to dampen microglial inflammation. Another target to inhibit the reactivity of retinal microglia is the sugar receptor Siglec 11. In humanized Siglec11 transgenic mice, we could show that a local treatment with Siglec 11 agonists could inhibit microglial reactions upon laser-damage and reduce vessel leakage as early sign for neovascular processes. In summary, we present two promising new targets to block overshooting microglial reactions in models for retinal degeneration.

IRB Status: None

Disclosures:

THOMAS LANGMANN, PHD: No financial relationships to disclose

O391

MECHANISMS OF PHOTORECEPTOR TOXICITY OF MONONUCLEAR PHAGOCYTES

XAVIER GUILLONNEAU, Shulong Hu, Bertrand Calippe, Sophie Lavalette, Florian Sennlaub

INSTITUT DE LA VISION

Age related macular degeneration (AMD) is associated with an accumulation of subretinal mononuclear phagocytes (MPs). In Geographic Atrophy, MPs accumulate in the vicinity of the atrophic lesion and are thought to contribute to photoreceptor degeneration by an unknown mechanism. Using animal models of subretinal inflammation, we have shown that subretinal MPs that originate from the blood circulation and that enter and accumulate in the subretinal space are responsible for photoreceptor loss. We here show that CX3CR1 deficiency resulted in an exacerbated neurotoxicity of subretinal MPs. Using an experimental model in which C57BL/6 or CX3CR1^{-/-} monocytes are allowed to differentiate into MP in the presence of retinal explants or photoreceptor outer segments, we present evidence that CX3CR1^{-/-} deficiency leads to the differentiation of subretinal MPs into an exacerbated pro-inflammatory profile. Inhibiting neurotoxic mediators produced by subretinally differentiated CX3CR1^{-/-} monocytes by pharmacological means efficiently reduces photoreceptor loss in vitro and in animal models of subretinal inflammation without affecting MP accumulation. Our result provides new rationales to protect retina from damaging age-dependent subretinal inflammation.

IRB Status: Approved

Disclosures:

XAVIER GUILLONNEAU, PHD: No financial relationships to disclose

O392

REGULATING MACROPHAGE PHENOTYPE AND FUNCTION BY RETINAL PIGMENT EPITHELIAL CELLS

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Purpose: We have shown previously that macrophages/microglia accumulate in the subretinal space and express CD68 and Arginase-1 in the aging eye. Subretinal macrophages are in close contact with retinal pigment epithelial (RPE) cells. We hypothesize that RPE cells may play an important role in regulating macrophage/microglial phenotype and function. The aim of this study was to investigate the effect of RPE cells on the phenotype and function of bone marrow-derived macrophages (BM-DMs).

Methods: BM-DM from C57BL/6J mice were cultured in DMEM supplemented with 20% L929 cell supernatant for 5 days. The phenotype of BM-DMs was confirmed by flow cytometry as CD11b⁺F4/80⁺. Primary RPE cells were cultured from C57BL/6J mice and confirmed by RPE65 and cytokeratin staining. BM-DMs were co-cultured with different types of RPE cells (healthy, oxidized, and apoptotic RPE) and then isolated from the co-culture system for phenotypic and functional assays.

Results: Co-culture of BM-DMs with RPE cells results in a time-dependent down-regulation of MHC-II expression and the generation of CD11b⁺F4/80⁺Ly6G⁺ myeloid-derived suppressor cells (MDSC). qRT-PCR analysis showed that RPE-induced MDSCs expressed high levels of IL-6, IL-1 β , and Arginase-1, but lower levels of IL-12p40 and TNF- α compared to naïve BM-DMs. The expression levels of iNOS, TGF- β and Ym1 did not differ

between naive BMDMs and RPE-induced MDSCs. Furthermore, functional studies showed that these cells had reduced phagocytic activity and lower ability to stimulate T cell activation and proliferation. When RPE cells were pre-treated with oxidized photoreceptor outer segments before co-culturing with BMDMs, the expression of IL-1 β and IL-6 in BMDMs was increased whereas the expression of Arginase-1 was decreased.

Conclusion: Our results suggest that healthy RPE cells can convert BMDMs into myeloid-derived suppressor cells under in vitro culture conditions, RPE-induced myeloid-derived suppressor cells are CD11b+F4/80+Ly6G+MHCII^{low}IL6+IL1b+Arg-1+. The ability of RPE cells is reduced when suffering from oxidative insults.

IRB Status: None

Disclosures:

MEI CHEN, PHD: No financial relationships to disclose

RC07 – Unfolded Protein Response in Oxidative Stress in Retinal Degenerations

O393

ROLE OF THE UNFOLDED PROTEIN RESPONSE IN REGULATION OF RPE CELL SURVIVAL

SARAH ZHANG

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The endoplasmic reticulum (ER) is the central hub for protein biosynthesis, protein folding and calcium storage. Maintaining a homeostatic environment in the ER is vital for normal cellular activities and survival. The retinal pigment epithelium (RPE) is a monolayer of pigmented epithelial cells located adjacent to photoreceptors. Damage to the RPE is a major pathological feature in several blinding diseases such as age-related macular degeneration. However, the molecular mechanisms that underlie the RPE injury remain largely unknown. Here, we show that the signaling pathways activated by ER stress, or the unfolded protein response (UPR), modulate the level of oxidative stress and apoptosis in RPE cells. Exposure of human RPE cells to condensate cigarette smoking extract (CSE) results in a dose- and time-dependent increase in ER stress and activation of UPR genes, accompanied by elevated reactive oxygen species (ROS) and mitochondrial fragmentation prior to apoptosis. Pre-treatment with anti-oxidant NAC or chemical chaperone TMAO markedly reduced ER stress, ameliorated intracellular ROS, improved mitochondrial morphology and protected cells from apoptosis. Overexpressing the anti-oxidant gene Nrf2 or the UPR effector XBP1 showed remarkable protective effects. In contrast, down-regulating these genes resulted in increased CHOP expression, enhanced caspase-3 activation and exacerbated cell death. Surprisingly, knockdown of CHOP, the well-known mediator of ER stress-related apoptosis, failed in protecting RPE cells but significantly increased the number of apoptotic cells and enhanced caspase-3 activation. Further investigation shows that loss of CHOP reduced the level of Nrf2 suggesting a feedback regulatory loop between

pro-apoptotic and anti-apoptotic molecules that may contribute to the fine-tuning of the apoptotic process. These results provide strong evidence of close interactions between cellular responses to oxidative stress and ER stress in RPE injury and further point to the functional complexity of the UPR signaling in regulation of RPE cell survival in pathological conditions.

IRB Status: None

Disclosures:

SARAH ZHANG, MD: No financial relationships to disclose

O394

THE ROLE OF ADVANCED GLYCATION/LIPOXIDATION IN THE PATHOGENESIS OF DIABETIC RETINOPATHY

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Retinopathy is a major cause of vision loss in patients with diabetes. The pathogenesis of this diabetic microvasculopathy is complex and is impacted by a raft of systemic abnormalities and activation of several aberrant pathways. One such pathogenic pathway is the formation and accumulation of advanced glycation end products (AGEs) and advanced lipoxidation end products (ALEs) which form at an accelerated rate in various cells in the diabetic retina. In this presentation, AGE/ALE formation in the diabetic retina will be reviewed alongside the impact of these adducts on retinal cell function. There is special emphasis placed on AGE/ALE-linked oxidative and immune-linked pathways in the retina. In unison with AGE/ALE formation there is a possible role of the receptor for advanced glycation end products (RAGE) in diabetic retinopathy. RAGE has been strongly linked to pro-inflammatory and oxidative pathology and the role of this receptor in microglial activation, neurophysiological and retinal microvascular pathology during diabetes will be discussed. In the future, new therapeutic agents will become available to prevent key biochemical and metabolic abnormalities that are definitively linked to neuroglial and vascular pathology. Growing evidence suggests that AGE/ALE formation and activation of RAGE are important and their inhibition and/or blockade of the downstream signaling pathways could be a useful therapeutic strategy to target the initiation and progression of diabetic retinopathy.

IRB Status: None

Disclosures:

ALAN STITT, PHD: No financial relationships to disclose

O395

ENDOPLASMIC RETICULUM-ASSOCIATED DEGRADATION (ERAD) OF MISFOLDED RHODOPSIN DISRUPTS PHOTORECEPTOR PROTEIN HOMEOSTASIS IN RETINAL DEGENERATION

JONATHAN LIN¹, Wei-Chieh Chiang¹, Heike Kroeger¹, Carissa Messah¹, Doug Yasumura², Michael Matthes², Sanae Sakami³, Judith Coppinger¹, Krzysztof Palczewski³, Matthew LaVail²

Rhodopsin is a G-protein coupled receptor that is essential for phototransduction by rod photoreceptor cells. Over 150 missense mutations in rhodopsin have been identified in the hereditary forms of retinal degeneration. Many rhodopsin missense mutations, such as P23H, cause opsin protein misfolding. P23H opsin causes endoplasmic reticulum (ER) stress and activates the Unfolded Protein Response (UPR) intracellular signaling network. However, the function of UPR in determining the fate of photoreceptors expressing misfolded opsin is unclear. Here, we examined a P23H opsin knock-in mouse that recapitulates the genetic and retinal degeneration phenotype seen in families. We find that the IRE1 signaling pathway of the UPR is strongly activated in photoreceptors expressing P23H rod opsin but not other retinal cell types. IRE1 activation leads to transcriptional upregulation of genes involved in endoplasmic reticulum-associated protein degradation (ERAD) in P23H retinas. ERAD preferentially targets P23H opsin for ubiquitination and rapid degradation, such that minimal opsin protein remains in photoreceptors. Opsin protein loss is evident in P23H animals as soon as photoreceptors are born and precedes photoreceptor cell death. By contrast to heterologous cell culture expression of misfolded opsin, virtually no mutant opsin aggregates within the ER of photoreceptors. Consistent with the efficient elimination of mutant opsin from the ER of photoreceptors, we find no significant increase in *Chop*, a gene strongly induced by persistent ER protein aggregation and chronic ER stress. Surprisingly, despite robust ERAD and rapid elimination of P23H rod opsin, total levels of ubiquitinated proteins markedly increase in photoreceptors during retinal degeneration. Our findings reveal highly efficient elimination of misfolded opsin protein from photoreceptors through the IRE1 signaling pathway of the UPR. We propose that buildup of damaged, ubiquitinated proteins is an unexpected consequence of ERAD of misfolded opsin leading to disruption of photoreceptor protein homeostasis and may contribute to retinal degeneration.

IRB Status: Approved

Disclosures:

JONATHAN LIN, MD, PHD: No financial relationships to disclose

O396

MODULATION OF THE UNFOLDED PROTEIN RESPONSE IN ADRP RETINA: POTENTIAL THERAPEUTIC IMPLICATIONS

MARINA GORBATYUK

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The PERK UPR signaling is activated in mouse retina expressing misfolded T17M rhodopsin (T17M). The PERK mediator pelf2a is known to promote up-regulation of ATF4 and pro-apoptotic CHOP proteins. Therefore, our goal is to manipulate the PERK signaling in ADRP photoreceptors in order to mitigate the retinal degeneration and validate new therapeutic targets. T17M, T17M CHOP^{-/-}, T17M ATF4^{+/-} and T17M CHOP^{+/-} mice were used in the study. Evaluation of the impact of CHOP ablation or ATF4 and CHOP deficiencies in ADRP photorecep-

tors was performed using ERG, SD-OCT, histology, qRT-PCR and western blot. Results of the scotopic ERG demonstrated that the ablation of CHOP protein in 1, 2, 3-month-old ADRP retina provoked a more severe form of retinal degeneration while the CHOP deficiency conversely mitigated retinal degeneration by from 72% to 38% up-regulation of the b-wave amplitudes. Interestingly, the ATF4 deficiency protected the T17M mice from retinal degeneration and induced significant rises in ERG amplitudes by over 2-fold. Results of the SD-OCT and histology were in agreement with the ERG data confirming functional and morphological protection of photoreceptors or expedite retinal degeneration. Western blot analysis demonstrated that protection of T17M ATF4^{+/-} photoreceptors occurred via reprogramming UPR and activation of anti-oxidant defense. The pATF6-50, CHOP and pelf2 were significantly lower but the HO-1 protein was considerably higher in T17M ATF4^{+/-} retina. Ablation of the CHOP, on the contrary, accelerated retinal degeneration through an 8-fold elevation of pelf2a suggesting transcriptional inhibition in ADRP retina. Our data demonstrated that the PERK signaling could be targeted in order to modulate the rate of retinal degeneration that strongly correlated with expression levels of the CHOP and ATF4 proteins. Increase in the CHOP or its ablation induced retinal degeneration. Reduction in the CHOP as well as in ATF4 promoted ADRP photoreceptor survival. Our data indicate pelf2a as a new therapeutic target.

IRB Status: Approved

Disclosures:

MARINA GORBATYUK, PHD: No financial relationships to disclose

O397

PROTECTION OF HUMAN RPE CELLS FROM ER STRESS BY HUMANIN: RELATIONSHIP TO ANTIOXIDANT ENZYMES AND MITOCHONDRIAL GSH

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DOHENY EYE INSTITUTE, KECK SCHOOL OF MEDICINE

Humanin (HN) is a recently discovered 24-amino acid mitochondrial peptide with neuro- and cardioprotective properties. Our aim was to study the effect of HN on ER-induced stress in fetal human RPE cells. Confluent primary human RPE cells (passages 2-4) were treated with tunicamycin (TM; 3 or 10 µg/ml) for 6-24 hours with or without HN (10 µg/ml) in serum-free culture medium. Confocal microscopy and western blot analysis were used to measure apoptosis (TUNEL and Caspase 3) and ER stress (GRP-78, CHOP and Caspase 4). Glutathione (GSH) biosynthesis was determined by gene and protein expression of the rate limiting enzyme γ-GCS (catalytic subunit) as a function of time. Levels of reduced GSH were measured in the total, cytosolic and mitochondrial compartments of RPE as a function of time. TM caused an increase in CHOP expression in RPE. ER-stressed RPE exhibited apoptosis as seen by an increase in caspase 3 (p<0.01 vs control), which was suppressed by HN. TM treatment showed a significant increase in γ-GCS mRNA at 6 and 12 hours (p<0.01 vs control). A significant increase in γ-GCS was also found when RPE were exposed to hydrogen peroxide-induced oxidative

stress. No appreciable changes in mRNA were seen with the antioxidant enzymes catalase, SOD-II, GRX-1 or TRX-1. The GSH/GSSG ratio decreased with TM, and showed a recovery with HN pretreatment. Treatment with HN alone caused an increase in mitochondrial GSH. In conclusion, our data indicate that HN protects RPE cells from ER-induced apoptosis and increases total and mitochondrial GSH in RPE cells. The protective action of HN may be linked to alterations in expression of anti-oxidative enzymes.

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IRB Status: None

Disclosures:

DOUGLAS MATSUNAGA, BA: No financial relationships to disclose

RC08 – Photoreceptor Cell Biology II

O398

RHODOPSIN TRAFFICKING PROTEINS AND RETINAL DEGENERATIONS

ALECIA GROSS, Evan Boitet, Nicholas Reish

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Precise vectorial transport of rhodopsin is essential for rod photoreceptor health and function. Mutations that truncate or extend the carboxy terminus of rhodopsin disrupt this transport and lead to retinal degeneration in human patients and in mouse models. We have found that such mutations disrupt the binding of rhodopsin to the small GTPase rab11a. The rhodopsin-rab11a interaction is a direct binding interaction that does not depend on the nucleotide binding state of rab11a. Expression of EGFP-rab11a fusion proteins in *Xenopus laevis* photoreceptors revealed that the nucleotide binding status of rab11a affects its subcellular localization, with GTP-locked mutants concentrated in the inner segment and GDP-locked mutants concentrated in the outer segment. ShRNA-mediated knock-down of rab11a in rods led to ectopic process formation and retinal degeneration. In the rab11a: rhodopsin complex, we detected an additional interacting protein that we identified as Nuclear Distribution C (NudC). Using bimolecular fluorescence complementation, we identified NudC: rab11a interactions at the Golgi and the base of the cilium in IMCD cells, and in the inner segment and ciliary axoneme of transgenic *Xenopus laevis* rod photoreceptors. Taken together our results show the critical importance of direct rhodopsin-rab11a interactions for formation and maintenance of vertebrate photoreceptors.

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IRB Status: None

Disclosures:

ALECIA GROSS, PHD: No financial relationships to disclose

O399

RHODOPSIN ACTIVELY RECRUITS THE SORTING MACHINERY THAT REGULATES ITS TRAFFICKING TO THE CILIA

DUSANKA DERETIC, Jing Wang

UNIVERSITY OF NEW MEXICO

The small GTPase Arf4 and the Arf GAP ASAP1 cooperatively regulate budding from the Golgi/TGN of the ciliary-targeted rhodopsin transport carriers (RTCs) that mediate membrane trafficking to the photoreceptor rod outer segments (ROS). We now report that during RTC budding Arf4 is activated by the Golgi-localized Arf guanine nucleotide exchange factor (GEF) GBF1. Treatment of retinas with Golgicide A (GCA), a selective inhibitor of GBF1, causes accumulation of rhodopsin in the Golgi and nearly completely blocks its transport to the ROS, much like the treatment with the pleiotropic Arf GEF inhibitor Brefeldin A (BFA). However, unlike BFA, GCA does not disassemble the Golgi. This suggests that in photoreceptors GBF1 regulates the export of rhodopsin from the Golgi, rather than the Golgi structure, which is likely controlled by other BFA-sensitive Arf GEFs. We find that rhodopsin actively recruits the sorting machinery that regulates its trafficking from the Golgi/TGN, through the formation of a functional complex with Arf4 and GBF1. The rhodopsin-Arf4-GBF1 complex is sensitive to GCA, pointing to the essential role of GBF1 in the activation of Arf4. Notably, GBF1 interacts in a GCA-resistant manner with ASAP1 at the Golgi/TGN and also on RTCs, despite their lack of Arfs. Thus, in the Golgi-to-cilia pathway the functional network of Arf4 surprisingly includes interactions of its GEF GBF1 with its GAP ASAP1. Our data show that the Golgi/TGN influx of rhodopsin, and potentially other ciliary cargo, is a vital signal that GBF1 recognizes to activate Arf4 and accordingly, with the input from ASAP1, control the Arf4 activation cycles during membrane trafficking to primary cilia.

IRB Status: None

Disclosures:

DUSANKA DERETIC, PHD: No financial relationships to disclose

O400

THE ROLE OF $Ca_v1.4$ CHANNELS IN THE DEVELOPMENT OF SYNAPTIC RIBBONS

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Neurotransmitter release from photoreceptor synaptic terminals requires signaling mediated by $Ca_v1.4$, a voltage-gated L-type Ca^{2+} channel. Perturbations in $Ca_v1.4$ can lead to a number of related visual disorders including congenital stationary night blindness, type 2 (CSNB2). $Ca_v1.4$ dysregulation in animal models causes reduced or absent neurotransmission; in addition, remodeling and degeneration of the synapse occurs. A hallmark of the photoreceptor synapse is

the ribbon, a dynamic organelle that participates in organizing the synaptic active zone. Components of the ribbon first appear in the terminal as precursor spheres that elongate into the mature form at the time of eye opening. Mature ribbons are absent when $\text{Ca}_v1.4$ is absent. It is not known if that is due to a failure in development or maintenance of the ribbon. We examined the development of ribbon synapses in multiple mouse models of CSNB2. In $\text{Ca}_v1.4$ KO retina the ribbon did not develop into the mature form. In mice with hypo- or hyper-active $\text{Ca}_v1.4$ channels, the ribbon was normal prior to eye opening but largely failed to mature. Exogenous expression of $\text{Ca}_v1.4$ channels using in vivo electroporation was sufficient to restore morphology of the synapse in $\text{Ca}_v1.4$ KO rods. Surprisingly, expression of a mutant channel lacking calcium conduction restored synaptic morphology. In the latter, ribbons were elongated but still shorter compared to those formed in the presence of functional $\text{Ca}_v1.4$. These data lead to a model in which $\text{Ca}_v1.4$ has a scaffolding role in the initial development of the ribbon synapse and proper regulation of its activity is required for maintenance.

IRB Status: Verified

Disclosures:

SHEILA BAKER, PHD: No financial relationships to disclose

O401

MULTIPLE FATES OF MUTANT P23H RHODOPSIN IN DEGENERATING PHOTORECEPTORS

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P23H is the most commonly occurring RP-causing rhodopsin mutation in the North American population. We have modeled this form of RP in transgenic *X. laevis* using human and bovine forms of P23H rhodopsin, which can be easily distinguished from the endogenous *X. laevis* rhodopsin using species-specific antibodies. On average, these animals express much less transgene product than comparable animals expressing WT rhodopsins, and there is prominent ER retention of the P23H rhodopsins, indicating that a biosynthetic defect is present. However, some P23H rhodopsin does exit the ER and is transported to the outer segment. The fraction of P23H rhodopsin that trafficks to the OS is negatively influenced by light exposure and vitamin A deprivation, both of which also increase the extent of retinal degeneration, indicating that one of the insults that drives retinal degeneration originates from P23H rhodopsin located in the inner segment. Electron microscopy indicates that retinal degeneration is associated with large increases in autophagy within the inner segment, possibly targeting inner segment accumulations of mutant protein. A portion of the P23H rhodopsin undergoes N-terminal cleavage, removing the H23 residue; this N-terminal truncated rhodopsin can be detected by cleavage-specific antibodies, and unlike the full-length protein, it accumulates only in outer segments, where it comprises the majority of the mutant rhodopsin present. In dark reared animals subsequently exposed to light, there

are immediate large increases in disorder of outer segment disks, suggesting instability of the mutant or cleaved mutant protein in outer segments. Our results suggest that there are multiple forms of P23H rhodopsin and multiple possible mechanisms underlying cell death. Environmental influences can result in enhanced trafficking or prevalence of certain forms, potentially promoting one mechanism of retinal degeneration while limiting another.

IRB Status: Approved

Disclosures:

ORSON MORITZ, PHD: No financial relationships to disclose

O402

RHODOPSIN-MEDIATED PHOSPHOLIPID FLIP-FLOP: IMPLICATIONS FOR A2E SYNTHESIS

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We recently showed that opsin, the rhodopsin apoprotein, is an ATP-independent flippase that equilibrates phospholipids across photoreceptor disc membranes. The mechanism by which opsin translocates phospholipids from one side of a membrane bilayer to the other is unknown. We now report that rhodopsin flips phospholipids independently of its light-sensing function: in addition to rhodopsin, M257Y-rhodopsin (an analog of transducin-activating metarhodopsin II), and two forms of opsin, representing structurally discrete conformational states of the protein were all able to facilitate rapid, ATP-independent flip-flop of phospholipid probes when reconstituted into large unilamellar vesicles. We are currently systematically disrupting lipid-facing surfaces in rhodopsin's transmembrane helices to determine whether these alterations affect lipid flipping. We propose that rhodopsin functions as a phospholipid "release valve" in disc membranes: it relieves transbilayer stress caused by the unidirectional lipid pumping activity of the ABC transporter ABCA4 and by so doing, it enables ABCA4 to function. As ABCA4 is intimately associated with the flux of precursors that generates the toxic bisretinoid A2E in retinal pigment epithelial cells, our results support the view that rhodopsin's flippase activity is a key determinant of A2E synthesis.

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IRB Status: None

Disclosures:

ANANT MENON, PHD: No financial relationships to disclose

RC09 – Cell Polarity and Signaling during Ocular Development

O403

REGULATION OF MITOTIC SPINDLE ORIENTATION AND ASYMMETRIC CELL DIVISIONS IN THE DEVELOPING MOUSE RETINA

MICHEL CAYOUE

IRCM

Asymmetric cell division (ACD) is a key process by which neural progenitors generate cell diversity. In the developing mouse retina, we previously reported that reorientation of the mitotic spindle along the apico-basal axis correlates with the production of terminal asymmetric cell divisions producing two neuronal daughter cells of different types. The molecular mechanisms controlling mitotic spindle orientation in retinal progenitor cells (RPCs), however, remain unknown. Work carried out mostly in invertebrate models has shown that the Gxi/LGN/NuMa complex connects the aster microtubules to the apical polarity complex (Par3/Par6/aPKC) to promote apico-basal divisions. In vertebrates, however, only a fraction of divisions occur within the apico-basal axis; most are actually oriented within the plane of the neuroepithelium, suggesting the existence of an elusive mechanism to negatively regulate the Gxi/LGN/NuMa complex. Using proteomics in mammalian cells, we identified the Suppressor APC Domain Containing 2 protein (Sapcd2) as a novel interactor of Gxi, LGN and Par3. In cultured MDCK cells, Sapcd2 overexpression delocalizes LGN from the cell cortex and leads to division orientation defects. In the developing mouse retina, Sapcd2 inactivation leads to a drastic increase in the proportion of apico-basal RPC divisions and a concomitant increase in terminal ACDs generating a photoreceptor and a bipolar cell, at the expense of terminal symmetric divisions generating two photoreceptors. Conversely, inactivation of LGN decreases apico-basal divisions, which leads to production of fewer terminal ACDs and more symmetric divisions producing two photoreceptors. Altogether, these results demonstrate the essential role of division orientation in regulating symmetric/asymmetric output in terminal divisions of the developing mouse retina, and identify Sapcd2 as a novel negative regulator of the Gxi/LGN/NuMa complex.

IRB Status: None

Disclosures:

MICHEL CAYOUE, PHD: No financial relationships to disclose

O404

LHX2 CHIP-SEQ ANALYSIS IDENTIFIES TARGET GENES CONTROLLING PROGENITORS MAINTENANCE AND LINEAGE COMMITMENT IN EARLY POSTNATAL MURINE RETINA

CRISTINA ZIBETTI, Jianfei Hu, Woonchang Hwang, David O'Brien, Hao Zhang, Jiang Qian, Seth Blackshaw

JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE

LHX2 has been shown to promote the maintenance of retinal progenitor cells (RPCs) regulating their transition towards a glial fate and the expression of neuro-protective factors in hypertrophic gliosis. Recent evidence proved LHX2 orchestrates diverse developmental programs by preserving cohorts of RPCs and preventing the precocious production of later cell types. To investigate LHX2 pleiotropic functions we comprehensively profiled LHX2 genome wide binding sites by Chip-sequencing in the murine retina at postnatal day 2 when 95% of LHX2 expressing cells are represented by RPCs and less than 5% by Müller glia precursors. Peak calling algorithms identified the 5'UTR and the promoters regions among the most represented categories over the genome. By adopting an extension-based association rule 50% of LHX2 binding sites fall distally from the TSS indicating LHX2 regulatory role is reflected but not restricted to direct promoters binding. Gene Ontology analysis on promoters revealed enrichment for retinal clusters, the Wnt signaling pathway and DNA repair corroborating LHX2 role in response to stress and repair mechanisms; retinal ganglion cells were found consistent with previous observations that LHX2 is required to constrain their production during a critical window. Camera-type eye, oligodendrocytes, and bipolars emerged from a binomial analysis of the extended regulatory domains. Among the identified targets enriched by qPCR and significantly moved in aged-matched flow sorted RPCs we found regulators of RPCs identity like the effectors of Notch and Fgf pathways, bHLH transcription factors, targets involved in glial reactivity and neurotransmitters homeostasis. LHX2 cofactors dependencies were inferred by motifs enrichment analysis revealing LHX2 consensus, NRF1, ETS family members and novel genomic instances including lim-homeodomain proteins, sox family members and factors involved in stemness maintenance. These results will ultimately provide insight into the mechanisms by which LHX2 regulates divergent processes at different retinal developmental stages.

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IRB Status: None

Disclosures:

CRISTINA ZIBETTI, PHD: No financial relationships to disclose

O405

POLARITY REGULATORS AND CANCER IN THE MURINE RETINA

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LUNENFELD TANENBAUM RESEARCH INSTITUTE

We work on the role of cell cycle regulators in retinal development and cancer. Using a conditional knockout approach we generated a model of retinoblastoma in which the Rb gene is deleted together with its relative p107 (*Cancer Cell* 2004, PMID:15193257). Normally, newborn differentiating neurons are post-mitotic, but Rb or Rb/p107 deficient retinal neurons continue to divide aberrantly due to unleashed E2f1 or E2f2 activity (*PLoS Biol* 2007 PMID:17608565, *Cell Death and Diff* 2013 PMID:23558950). In unpublished work we noted that the tumor-prone Rb/p107 retina also has a defect

in apical polarity, which is detectable in the early embryonic tissue. Notably, this defect is not observed in the tumor-resistant Rb null retina, suggesting that together with the cell cycle defect, disruption of apical polarity may also play a role in tumorigenesis. I will discuss our progress on trying to understand the link between cell cycle regulators, polarity disruption and cancer initiation.

IRB Status: Approved

Disclosures:

ROD BREMNER, PHD: No financial relationships to disclose

O406

DISTINCT DISTRIBUTIONS AND FUNCTIONS OF THREE CRUMBS (CRB) PROTEINS IN THE ZEBRAFISH RETINA

XIANGYUN WEI

UNIVERSITY OF PITTSBURGH

Numerous apicobasal polarity proteins are required for the polarity, integrity, and morphogenesis of the retina. Among these polarity proteins are the evolutionarily conserved Crumbs (Crb) proteins. Interestingly, three highly similar Crb proteins (Crb1, Crb2a, and Crb2b) are expressed in surprisingly varying patterns in the zebrafish retina. Therefore, it is important to thoroughly understand the differences in Crb expression patterns, the mechanisms underlying such varied expressions, as well as any resulting divergences in Crb functions. Here we present our progress on these issues. First, we show that the three Crb proteins are expressed in cell-type-specific and organelle-specific manners: Crb1 localizes to the outer segment (OS) of all types of cone photoreceptors; Crb2a localizes to the subapical regions (SARs) of the inner segment (IS) of both rod and cone photoreceptors as well as to the apical processes of Müller glial cells; and Crb2b only localizes to the SARs of the green, red, and blue cones but not to that of the UV cones and rod photoreceptors. Second, we identify a previously unrecognized Crb intracellular motif (referred to as PDZ-binding regulator) which regulates differential targeting of Crb between the IS and the OS, likely by adjusting the binding affinities between Crb's PDZ-binding motifs and Crb's cytoplasmic PDZ partners. Third, we demonstrate that Crb2a and Crb2b mediate intercellular adhesions at the cone SARs through their extracellular domains; such adhesions are required for proper cone patterning and cone survival in the zebrafish retina. These findings offer novel insight into the basic biology of Crb functions in zebrafish retinal development and maintenance; moreover, these findings facilitate our understanding of human retinal health and diseases, such as CRB1-related retinitis pigmentosa.

IRB Status: None

Disclosures:

XIANGYUN WEI, PHD: No financial relationships to disclose

RC10 – Signaling Sphingolipids in Ocular Diseases

O407

SPHINGOLIPID-MEDIATED INFLAMMATION AT THE OCULAR SURFACE

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NATIONAL INSTITUTE OF HEALTH AND WELFARE, PUBLIC GENOMIC RESEARCH UNIT²

Sphingolipids (SLs) are indispensable lipids that influence cellular responses through their effects on membrane biophysical properties or direct interaction with target proteins. Their biological significance is increased by variability and adaptability as there are tens of enzymes designed to modulate their function. Our research aims to elucidate if and how the regulatory potential of the signaling SLs is harnessed in corneal stress response.

Cellular stress response concludes with either inflammation or cell death, depending on the strength of the stimulus, and both paths have a crucial impact on the health of the tissue. We found that the SL signaling pathway is activated in human corneal epithelial (HCE) cells by numerous stimuli relevant to the ocular surface (LPS, UV-B radiation and hyperosmolarity). The activation of the SL pathway concludes with the secretion of IL-8, an important mediator of the immune response at the ocular surface. Moreover, the above-mentioned stimuli induce dose dependent release of SL enzymes into the extracellular environment and these enzymes were identified in human tear samples as well. Interestingly, the SL enzymes are released from HCE cells associated with extracellular vesicles that have the capacity to moderate the inflammatory response of corneal epithelial cells.

The SL metabolism is presently acknowledged as an important pathway in cellular stress response, and our work focuses on its particularly appealing potential in the management of ocular inflammation.

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IRB Status: Approved

Disclosures:

ALEXANDRA ROBCIUC, MSC: No financial relationships to disclose

O408

SPHINGOSINE-1-PHOSPHATE SIGNALING IN RETINA PHOTORECEPTORS AND GLIAL CELLS

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INSTITUTE OF BIOCHEMICAL RESEARCH, UNS-C, DEPARTMENT

Sphingosine-1-phosphate (S1P) is a potent sphingolipid mediator that regulates proliferation, survival, migration and inflammation in different cell types, acting on S1P membrane receptors (S1PRs) or as an intracellular messenger. S1P roles in the retina are still poorly understood. We have demonstrated that S1P promotes proliferation and differentiation in photoreceptors and rescues them from oxidative stress-induced apoptosis. We here investigated the pathways leading to S1P protective effect. Pretreatment of neuronal cultures with a S1PR antagonist or with PD98059, an ERK/MAPK pathway inhibitor, abolished S1P protection from oxidative stress, while LY294002, a PI3 kinase inhibitor, had no effect. This implies S1P activates S1PRs in photoreceptors, which then promote photoreceptor survival through the ERK/MAPK pathway.

Since we have shown that Müller glial cells protect photoreceptors from oxidative stress-induced apoptosis, we explored whether S1P was involved in this protection. In neuro-glial cultures grown in S1P-lacking media, addition of an inhibitor of S1P synthesis or an S1PR antagonist blocked glial protection, suggesting glial cells synthesize and release S1P to promote photoreceptor survival.

We also studied whether S1P regulated proliferation and migration of Müller glial cells, known to be involved in proliferative retinopathies. S1P enhanced glial proliferation and inhibiting the PI3K pathway blocked this effect. S1P also induced glial cell migration; S1P addition prompted formation of lamellipodia in glial cells, which then rapidly migrated in a scratch-wound assay. A S1PR antagonist or LY294002 blocked this migration, while PD98059 and SB203580, a p38 MAPK inhibitor, partially prevented it. Hence, S1P promotes glial proliferation and migration through activation of different intracellular pathways.

As a whole, our data point to a central role for S1P in the control of crucial processes in both photoreceptors and glial cells. Since deregulation of these processes is involved in several retinal pathologies, S1P signaling emerges as a potential tool for treating these diseases.

IRB Status: Verified

Disclosures:

NORA ROTSTEIN, PHD: No financial relationships to disclose

O409

COMPARISON OF VITREOUS AND SERUM SPHINGOLIPIDS IN PATIENTS WITH PROLIFERATIVE DIABETIC RETINOPATHY TO PATIENTS WITHOUT RETINAL VASCULAR DISEASE

LOUIS GLAZER¹, Todd Lydic², Julia Busik², Gavin Reid²

VITREO-RETINAL ASSOCIATES, P.C. OPTHALMOLOGY¹; MICHIGAN STATE UNIVERSITY²

Altered sphingolipid metabolism has been demonstrated to play an important role in the pathogenesis of diabetic retinop-

athy in animal model studies. The effect of diabetes on retinal sphingolipid metabolism in diabetic patients has never been reported. This study was designed to examine the effect of diabetes on sphingolipid profile of vitrectomy specimens.

We compared 7 vitreous samples from patients with proliferative diabetic retinopathy (PDR) to 6 vitreous samples from control patients with macular hole and macular pucker, but no retinal vascular disease. Additionally, serum was obtained from 3 PDR and 3 control patients in this cohort of 13 patients. All vitreous specimens were obtained from clinically indicated vitrectomies. Patients were required to sign written informed consent, and IRB approval was obtained for the research protocol. Lipid extracts from vitreous and serum samples were subjected to sphingolipid analysis using a combination of Orbitrap and triple quadrupole mass spectrometry and tandem mass spectrometry. Total vitreous sphingolipids were increased 30-fold in PDR patients compared to controls, with concomitant alteration of sphingolipid class distribution including a relative increase in sphingomyelin and relative decreases in ceramide, hexosylceramide, and lactosylceramide. Significant remodeling of vitreous sphingolipid molecular species were observed in PDR, including increased levels of long chain fatty acids and decreased levels of short chain fatty acids incorporated into sphingomyelin and ceramide, as well as a trend toward decreased very long chain (>C24) fatty acid-containing species of ceramide. None of the observed changes in vitreous sphingolipid content were recapitulated in serum from PDR patients, indicating that alterations in vitreous sphingolipid content likely reflect diabetes induced disruption of retinal, rather than systemic, sphingolipid metabolism.

IRB Status: Approved

Disclosures:

LOUIS GLAZER, MD: No financial relationships to disclose

O410

OVEREXPRESSION OF ELOVL4 PREVENTS DIABETES-INDUCED BLOOD-RETINAL BARRIER BREAKDOWN THROUGH AN INCREASE IN VERY LONG CHAIN CERAMIDES

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MICHIGAN STATE UNIVERSITY¹; UNIVERSITY OF MICHIGAN, KELLOGG EYE CENTER²; UNIVERSITY OF FLORIDA³

Diabetes induced loss of the blood-retinal barrier occurs early in the progression of diabetic retinopathy and contributes to macular edema and vision loss. Several mechanisms affecting vascular permeability in the diabetic retina have been identified including vascular endothelial growth factor alterations in junctional complex organization and content; however, the role of retinal lipid changes in permeability has not been studied. Maintenance of water permeability barrier in skin requires very long chain (VLC, \geq C26) ceramides and ELOVL4 is the elongase responsible for production of VLC fatty acids that are incorporated into VLC ceramides. We have previ-

ously demonstrated a decrease in ELOVL4 in diabetic retina. The goal of this study is to determine whether restoration of retinal ELOVL4 levels is sufficient to prevent diabetes induced increase in retinal permeability. Streptozotocin (STZ) diabetic rats received intravitreal injection of hELOVL4 packaged in adeno-associated virus type 2 containing four capsid Y-F mutations (AAV2 mut quad) under the control of a small chicken β -actin (smCBA) or vehicle. Retinal vascular permeability was assessed by measuring extravasation of FITC-albumin after 8 weeks of diabetes. Human ELOVL4 was overexpressed in human retinal pigmented epithelial (RPE) cells or bovine retinal endothelial cells (BREC) by an E1- and E3-deleted adenoviral vector. Lipid extracts were analyzed by tandem mass spectrometry. Permeability was determined in confluent monolayers by RITC dextran. Intravitreal delivery of hELOVL4-AAV2 in STZ diabetic rats resulted in a 39% reduction in diabetes-induced vascular permeability after 8 weeks of diabetes. Overexpression of hELOVL4 in RPE and BREC caused a 37% and 33.2% reduction in permeability respectively. Lipidome analysis of hELOVL4-overexpressing cells revealed a 2.5-fold increase in 26:0 ceramide relative to controls, while 16:0 ceramide decreased 1.7-fold.

In conclusion, normalization of retinal ELOVL4 expression could prevent early breakdown of the blood–retina barrier in diabetic animals by modulating retinal sphingolipid metabolism.

IRB Status: None

Disclosures:

NERMIN KADY, PHD: No financial relationships to disclose

O411

REVERSE GENETICS TO UNDERSTAND THE ROLE OF SPHINGOLIPID IN THE RETINA

NAWAJES MANDAL, Madeline Budda, Hui Qi, Megan Stiles, Tuan-Phat Huynh, William Johnson

UNIVERSITY OF OKLAHOMA HEALTH SCIENCES CENTER

Sphingolipids are essential components of every cell membrane and are especially important for development and maintenance of neural tissues. Many sphingolipid metabolites, such as ceramide (Cer), sphingosine (Sph) and sphingosine-1-phosphate (S1P) are bioactive lipids that act as second messengers to regulate cellular functions ranging from apoptosis to inflammation, cell adhesion, migration and neovascularization. Apoptotic photoreceptor cell death is the ultimate irreversible cause of blindness in many forms of human retinal degeneration. Furthermore, inflammatory retinal diseases presently account for the largest group of patients with retinal dystrophy, including age-related macular degeneration (AMD), diabetic retinopathy and posterior uveitis.

We have characterized genetic mouse models of sphingosine kinase (Sphk) 2 knock-out (Sphk2 KO), S1P receptor 2 knock-out (S1P2 KO) and retina-specific conditional KO of Acyl sphingosine amido-hydrolase (Asah1, acid Ceramidase) gene by subjecting them to electroretinography (ERG) to study function of the rod and cone photoreceptors, fundus photography, fluorescein angiography, OCT and histological analyses. In this reverse genetics approach, we identified for the first time RPE-choroidal pathology in Sphk2 and S1P2 KO mice. Sphk2 KO mice also showed enhanced cone functionality and the protein SPHK2 showed light-dependent movement to the photoreceptor outer segment. We found that conditional deletion of acid Ceramidase (Asah1) gene in the retina affects the ganglion cell layer. The ganglion cell loss is associated with increased ocular pressure.

Our study will potentially identify novel genes, pathways for eye diseases, provide models to study the mechanism and identify novel targets for therapies. Ceramide has been associated with inflammation and apoptotic neural cell death. We believe the accumulation of ceramide in Asah1 conditional KO mice retina induces microglial activation, inflammation and ganglion cell death. This could serve as a useful model of Glaucoma.

IRB Status: None

Disclosures:

NAWAJES MANDAL, PHD: No financial relationships to disclose

Joint Sessions

JT01 – RPE and Photoreceptor Biology

O412

SMALL PEPTIDES FROM PEDF THAT BIND PEDF-R AND PROTECT THE RETINA

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NIH-NEI¹; UNIVERSITY OF MODENA AND REGGIO EMILIA²

Pigment epithelium-derived factor (PEDF) protects the retina against degeneration. A peptide region toward the N-terminus (44mer) of PEDF confers neurotrophic activity to the protein. We identified a PEDF receptor, PEDF-R, which is crucial for the PEDF retinoprotective effects and mapped its ligand binding domain to a central region of the human PEDF-R (positions 203-232). However, the receptor binding domain on PEDF remains to be identified. This study aims to map the region on PEDF that binds PEDF-R to exert retinoprotective activity. Peptides from the human PEDF sequence (34mer, positions 44-77; 44mer, 78-121; 17mer, 98-104; and sixteen 17mer peptides with single amino acid alterations [alanine scan]) and from human PEDF-R (P1, 210-249) were chemically synthesized. Peptide 44mer bound to full-length human PEDF-R and P1, whereas 34mer did not bind, by ligand blotting and pull-down assays. The 17mer also bound P1. Fluorescent anisotropy revealed that while 17mer with an alteration at arginine 99 to alanine (R99A) did not change the polarization of FITC-conjugated P1, 17mer[H105A] increased it relative to that with the unmodified 17mer, implying a higher affinity for 17mer[H105A]. Apoptosis was induced in rat retina R28 cells by serum starvation. Full-length human PEDF and 44mer, 17mer and 17mer[H105A] decreased the number of TUNEL-positive nuclei in R28 cells, but 17mer[R99A] and 34mer had no antiapoptotic effect. Peptides were injected intravitreally into *rd1* mice at PD11 and photoreceptor survival was assayed a day later. PEDF and peptides 44mer, 17mer and 17mer[H105A], except 17mer[R99A], decreased the number of TUNEL-positive nuclei of photoreceptors. Additions of molar excess of P1 (blocking peptide of PEDF/PEDF-R interactions) over PEDF hindered the effects of the active peptides. Thus, a small region within the 44mer of PEDF is retinoprotective and contains a novel PEDF-R binding site, implying that their interactions could trigger downstream effects for protecting photoreceptors from degeneration.

IRB Status: None

Disclosures:

S. PATRICIA BECERRA, PHD: No financial relationships to disclose

O413

IDENTIFICATION AND CHARACTERIZATION OF THE TRANSMEMBRANE RECEPTORS FOR A SECRETED FACTOR WITH BROAD THERAPEUTIC VALUES

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UCLA, SCHOOL OF MEDICINE

Pigment Epithelium Derived Factor (PEDF) was first identified as a neurotrophic factor secreted by the retinal pigment epithelium (RPE) cells in the eye more than 20 years ago. Through unbiased searches, it was also later identified as one of the most potent, if not the most potent, antiangiogenic factors, a stem cell niche factor, an anti-inflammatory factor and a factor that directly inhibits tumor growth. It has potentially broad therapeutic values in various human diseases especially in vision diseases. Uncovering the cell-surface receptors responsible for these activities and their transmembrane signaling pathways can lead to a better understanding of the mechanisms of its diverse biological activities and the design of new therapeutic agents based on these mechanisms. After seven years of search using a variety of techniques, we identified two cell-surface transmembrane receptors for PEDF. These two receptors do not belong to well-known receptor families such as G-protein coupled receptors or receptor tyrosine kinases. One receptor is more restrictedly expressed in cells such as specific types of endothelial cells and the macrophage. The other receptor is more broadly expressed in endothelial cells and neurons. We have also identified another transmembrane domain protein that associates with these two receptors and regulates their cellular activities. This presentation will present our findings in the mechanistic studies of these two transmembrane receptors.

IRB Status: Verified

Disclosures:

HUI SUN, PHD: No financial relationships to disclose

O414

LOSS OF RETINITIS PIGMENTOSA 2 (RP2) PROTEIN MODULATES MICROTUBULE DYNAMICS AND CONE PHOTORECEPTOR OUTER SEGMENT EXTENSION

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UNIVERSITY OF SALAMANCA⁴

Loss of daytime vision due to defects in cone photoreceptors results in debilitating blinding disorders and poor quality of life. The light-sensing ciliary outer segment (OS) of photoreceptors is formed by extension of microtubule-based ciliary

axoneme. Due to overwhelming majority of rods (>95% rods and 3-5% cones) in mammals, it is not clear how cone photoreceptors undergo dysfunction and degeneration during disease. Here we show that the RP2 protein, which is mutated in X-linked photoreceptor degenerative diseases, predominantly affects cone photoreceptor function and morphology. We show that *Rp2^{null}* mice exhibit elongation of cone OS, as determined by immunofluorescence and transmission electron microscopy. This effect was phenocopied when the *Rp2* gene is conditionally inactivated specifically in cones, indicating that the effect of *Rp2* ablation on cones is cell autonomous. The long cone OS shows enrichment of microtubule-associated proteins, including acetylated α -tubulin, RP1, and kinesin subunit KIF17, but not of KIF3A. Furthermore, *Rp2^{null}* mouse embryonic fibroblasts (MEFs) develop longer cilia. This was associated with mild increase in soluble tubulin in the *Rp2^{null}* MEFs. These studies suggest that RP2 modulates cone outer segment extension, likely by influencing tubulin incorporation into axoneme and provide novel insights into the pathogenesis of loss of daytime vision in retinal degenerative diseases.

IRB Status: None

Disclosures:

HEMANT KHANNA, PHD: No financial relationships to disclose

O415

VARYING PATHOBIOLOGY FOR PRPH-2-ASSOCIATED DISEASE

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Mutations in the photoreceptor-specific gene peripherin-2 (*PRPH-2*, also known as retinal degeneration slow/*RDS*) result in widely varying, dominantly inherited retinal degenerations including retinitis pigmentosa (RP), cone-rod dystrophy (CRD), and various macular/pattern dystrophies. Historically, rod-dominant diseases (e.g. RP) were thought to arise from loss-of-function mutations and haploinsufficiency (caused by the mutant protein being unstable/degraded). Cones are more sensitive to subtle changes in RDS oligomerization than rods, and cone-dominant diseases such as CRD and macular dystrophy were thought to arise due to gain-of-function mutations which resulting in a stable but altered protein product. However, these explanations did not account for the vast phenotypic heterogeneity or for the development of RPE defects and choroidal defects which so often present in these patients. Therefore we generated knockin mouse models carrying pattern dystrophy/macular dystrophy mutations. Often, these mutations cause consistent loss-of-function phenotypes in both rod and cone photoreceptors including retinal degeneration, and ERG phenotypes similar to the *rd5^{+/+}* RP model. However, the same animals also exhibit gain-of-function phenotypes such as the accumulation of stable but abnormal RDS/ROM-1 oligomers which do not support proper OS formation, and the development of late-onset choroidal and RPE defects which manifest with incomplete penetrance. These findings have led us to hypothesize a third disease mechanism: namely that the formation of abnormal RDS oligomers, which are present but

non-functional in the photoreceptor OSs, can result in toxicity in the adjacent RPE and choroid (possibly in a similar way to the *abca4^{-/-}*). This toxicity would primarily affect the macula due to the metabolic demands on the RPE in that region. These exciting data suggest that any rational therapeutic treatment for RDS-associated macular/pattern dystrophies will need to address not only the presence of abnormal RDS oligomers in photoreceptors, but also overcoming secondary sequelae in neighboring tissues.

IRB Status: None

Disclosures:

MUNA NAASH, PHD: No financial relationships to disclose

O416

THE CELL POLARITY PROTEINS CRB2 AND CRB3 ARE EXPRESSED IN THE RPE

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Most of the retinal pigment epithelial (RPE) cells functions are performed thanks to its cell polarity. Two protein complexes provide the apical polarity to polarized cells, the CRB and PAR complexes. Some of the members of these complexes have been detected in the RPE but little is known about their functions in this tissue. There are 3 CRB protein isoforms in mammals, CRB1, CRB2 and CRB3; and all of them are expressed in the retina. They are all transmembrane proteins which share a highly evolutionary conserved cytoplasmic domain that binds several adapter and effector proteins conforming the CRB complex. This complex is located at the tight junctions, and determine the apical region of the cell. Although some of the CRB complex members have been detected in the RPE, none of the CRB proteins have been found in this tissue to date. In order to study the expression of these proteins in the RPE we have generated and characterized two new antibodies to distinguish CRB2 and CRB3 proteins. We have then determined the expression and location of these proteins as well as those of the PAR complex in the mouse RPE and in cultures of human RPE polarized cells. The human RPE polarized cells show a high polarization degree in culture, expressing specific polarity molecules as well as microvilli and melanosomes, being then a reliable and useful model to study the mechanisms of the polarization process. The antibodies designed by our group specifically recognize CRB2 and CRB3 proteins located at the tight junctions of the mouse RPE and in human RPE cells. The PAR complex is also present in the RPE and its appearance is earlier than of the Crumbs complex during the process of cell polarization. The role that these complexes play in the RPE remains to be elucidated.

IRB Status: Approved

Disclosures:

ANTONIO ESCUDERO, PHD: No financial relationships to disclose

OCULAR ALBINISM: INSIGHTS ON THE EXPRESSION OF RETINAL GENES REGULATED BY OA1

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Our purpose is to define the differential gene expression profiles associated with embryonic retinal cells from ocular albino Oa1^{-/-} mice and their corresponding control NCrI mice. It has been suggested that reduced/absent pigmentation in the RPE is associated with abnormal decussation of ganglion cell (RGC) axons at the optic chiasm but how RPE pigmentation controls this is unknown. In adult mice, the RPE and RGCs are at opposite ends of the retina, but during embryonic development, RPE cells could be in direct contact with RGC progenitors, regulating axons' guidance. Since melanin biosynthesis seems to play a role in the ipsilateral/contralateral decision of ganglion cell axons, differential hybridization of genes from control and diseased retinas in microarrays could identify those genes influencing RPE pigmentation and, directly or indirectly, RGCs axonal development and decussation. Eyes from E13 and E15 mouse embryos (7-13/litter) were collected from Oa1^{-/-} and NCrI pregnant females. Total RNA was isolated from all eyes of each litter and used as one biological sample. Three biological samples from each E13 and E15 Oa1^{-/-} and control samples were hybridized to 430.20 Affymetrix Mouse arrays. qPCR and immunocytochemistry were used to confirm the differential expression of selected genes. Two down-regulated genes were expressed in both E13 and E15 samples: Oa1, corroborating the validity of the microarray results, and Erdr1. The number of genes differentially expressed varied enormously from E13 to E15, with only 4 down-regulated genes at E13 and 47 down-regulated and 4 up-regulated genes at E15. In addition to Erdr1, we focused our studies on 2 other differentially expressed genes in E15 Oa1^{-/-} and control retinas, Dcx and Tfap2b, which may be potentially involved in normal/abnormal axonal development, neuroprotection and guidance to the brain. These genes may also influence normal melanogenesis and the abnormal pigmentation associated with ocular albinism.

IRB Status: None

Disclosures:

ALEJANDRA YOUNG, PHD: No financial relationships to disclose

αB-CRYSTALLIN REGULATES EXOSOMES SECRETION IN RETINAL PIGMENT EPITHELIAL CELLS

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The small heat shock protein αB-crystallin is associated with a wide range of neurodegenerations including Alzheimer's disease, Parkinson's disease, multiple sclerosis, cardiomyopathies, breast cancer and age-related macular degeneration (AMD). This protein has been reported to have anti-aggregation properties; it is anti-apoptotic, anti-inflammatory and neuroprotective. We have previously reported on the secretion of this protein via exosomes from the adult human retinal pigment epithelial cells in culture (ARPE-19). Exosomes contain cell-type specific cargo (RNAs and proteins) for delivery to extracellular destinations. Based on the assumption that the biogenesis of the exosomes is regulated by tissue physiology we believe that the exosomes may be assembled in response to molecular cues received from the surrounding tissues and cells. We therefore believe that the exosomal cargo may have an important role in exosomal biogenesis. Because the human retinal pigment epithelium straddles the blood/retina barrier, both physically as well as functionally, we tested the role of αB-crystallin in the secretion of exosomes employing the human RPE paradigm. Exosomes (proteolipid vesicles, ~150 nm in size) are synthesized as a part of the endosome pathway for protein homeostasis, an important function of the RPE physiology. We used shRNA to inhibit the expression of αB-crystallin in these cells; this leads to the arrest of exosomal secretion from these cells. Employing biochemical fractionation and electron microscopy we demonstrate that the inhibition of the exosome secretion is bought about by interruption of the progression of the multivesicular body (MVB) fusion with the plasma membrane. These observations have important implication in the functional state of the RPE in health and in disease.

IRB Status: International

Disclosures:

SURAJ BHAT, PHD: No financial relationships to disclose

DJ-1 EXPRESSION LEVELS PERTURB MITOCHONDRIA STRUCTURE IN THE RETINAL PIGMENT EPITHELIUM (RPE)

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CLEVELAND CLINIC

We have shown that in RPE cells under oxidative stress DJ-1 displays increased colocalization with mitochondria-specific antibodies. Therefore, we analyzed the effects of DJ-1 expression in the mitochondrial structure of RPE cells. ARPE-19 and mouse primary RPE cultures from control and DJ-1 knockout (KO) mice were treated with H₂O₂ (100 μM) for various times. In addition, cells were infected with adenoviruses carrying the full-length human DJ-1 cDNA (hDJ) and a mutant construct, which has its cysteine (C) residues mutated to serine (S) prior to stress experiments. A parallel group of cells was transfected with shRNA DJ-1 plasmids. Finally, RPE from DJ-1 KO and control mice were analyzed. Results were evaluated by electron microscopy, immunofluorescence and Westerns using mitochondria-specific antibodies. Oxidative stress produced a fragmented phenotype in the RPE cells mitochondria. A similar phenotype was observed in RPE cultures overexpressing both hDJ and C mutant. Mitochondria were more spread through the cytoplasm in RPE

cells transfected with control shRNA than in RPE cells transfected with DJ-1 shRNAs. Mitochondria in the RPE from control mice displayed individualized outer and inner membranes, intact cristae and matrix. In contrast, DJ-1 KO mice RPE displayed loss of cristae and increased matrix density. Labeling with COX IV and Tom20 antibodies revealed decreased signal of these mitochondrial proteins in the DJ-1 KO RPE. Next, an analysis of the expression of 7,8-dihydro-8-oxoguanine (8-oxoG) and COX IV was performed. In control mice, 8-oxoG labeling was observed below the RPE cells while it was elevated and present in the RPE cell bodies in the DJ-1 KO retina, which colocalized with COX IV labeling. Our results suggest that mitochondrial morphology is altered with the loss and increased expression of DJ-1. The mitochondria of DJ-1 KO RPE cells *in vivo* display: altered ultrastructure, decreased mitochondrial mass, and increased DNA oxidation.

IRB Status: None

Disclosures:

VERA BONILHA, PHD: No financial relationships to disclose

JT02 – Ion Channels in Eye Disease

O420

ION CHANNELS IN CORNEAL SENSORY INNERVATION: ROLE IN OCULAR PATHOLOGIES

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Sensory nerve terminals in the eye anterior surface detect external stimuli producing innocuous and painful sensations. Ion channels present in nerve terminals play a key role in sensory detection and their expression and properties undergo profound changes in conditions such as dry eye, chronic inflammation or ocular surface lesions. In a dry eye animal model, where basal tearing was reduced by exorbital removal of the main lacrimal gland, ocular surface wetness produced an increased firing of corneal cold thermoreceptor neurons and a reduction of the cold threshold. This increased excitability was the consequence of an enhancement of TTX-sensitive Na⁺ currents combined with a reduction of voltage-activated K⁺ currents. Enhanced activity of corneal cold sensory terminals is likely to be responsible for the dryness sensations accompanying dry eye disease. In addition to temperature sensing, sensory neurons in the ocular anterior surface are subject to significant pH changes. Moderate acidic pH (6.6) activated ASIC-like currents in corneal polymodal sensory neurons, which were blocked by ASIC1 or ASIC3-specific toxins. Acidic pH depolarizes corneal sensory neurons to fire action potentials, an effect blocked by APETx2, an ASIC3 blocker. Moderate acid stimulus (pH 5-6) produced nocifensive behaviors (blinking, scratching)

that were prevented by ASIC blockers. In a model of allergic keratoconjunctivitis, nocifensive behavior was greatly reduced by ASIC3 blockade, likely reducing the inflammatory process. These results show that ASICs, together with TRPV1 and TRPA1, play a significant role in the detection of acidic insults. The identification of new channels in corneal neurons as well as their alterations in different ocular pathologies are critical for understanding sensory physiology and may represent novel targets for the development of new therapeutic agents.

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IRB Status: International

Disclosures:

XAVIER GASULL, PHD: No financial relationships to disclose

O421

MECHANOSENSITIVE CHANNELS OF TRABECULAR MESHWORK

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Impeded aqueous humor outflow results in aqueous humor accumulation and elevated intraocular pressure (IOP) in primary open angle glaucoma. We postulated that trabecular meshwork (TM) cells actively sense surrounding shear stress and undergoes modulation involving following three steps: (1) an active mechanosensing step in the solution phase, (2) mechanotransduction of mechanosensing at the cell surface, and (3) cytoskeletal changes in response to mechanotransduction resulting in altered TM cell shape and motility. A key question remaining is what mechanosensitive channels are present on the TM surface and what are their paired mechanosensing molecules.

Human TM tissue, kidney extract, Primary TM cells, was used for western blot, immunohistochemical and mass spectrometric analyses following established protocols. Imaging was performed using a Leica TSP5 confocal microscope. Reciprocal immunoprecipitation (IP) and western blotting was performed using antibodies to the following channel proteins: Trek-1, Task-1, Piezo1, Piezo2, Trpa1, Trpc1, Trpp2, Trpc2, Trpc3, Trpc6, Trpm2 and cochlin. Whole-cell patch clamp was used for electrophysiological experiments in HEK293T transfected cells.

Our investigation have identified the presence of the following channels: Trek-1, Task-1, Piezo1, Piezo2, Trpa1, Trpc1, Trpp2, Trpc2, Trpc3, Trpc6, Trpm2 at protein level in the TM tissue and on isolated primary TM cells. Except for Piezo2, Trpa1, Trpc1, Trpc2, Trpc3 and Trpm2 we found cochlin to interact with all other channels present in the TM noted above using reciprocal IP. Our initial electrophysiological experiments using multimeric

and monomeric cochlin suggest functional consequences for cochlin-Trek-1 interaction in HEK293T cells expressing Trek-1.

We report the presence of several mechanosensitive channels in the TM. Cochlin interact with Trek-1 both in monomeric and multimeric forms leading to changes in the electrophysiological properties of Trek-1. It is likely that these interaction events effect downstream cytoskeleton changes in response to fluid shear and likely modulate fluid flow mediated changes in TM cells properties.

IRB Status: None

Disclosures:

SANJOY BHATTACHARYA, M TECH, PHD: No financial relationships to disclose

O422

RETINAL CNG CHANNELOPATHIES: FROM MECHANISMS TO TREATMENTS

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LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

Phototransduction in rod and cone photoreceptors relies on the function of cyclic nucleotide-gated (CNG) channels. The main functional role of these ion channels is to translate light-triggered changes in the cGMP levels into an electrical signal that is further processed within the retinal network and then sent to higher visual centers. Rods and cones express distinct CNG channels in their outer segments. Mutations in the genes CNGB1 and CNGB3 encoding the rod channel subunits result in retinitis pigmentosa while mutations in the cone channel subunits CNGB3 and CNGB3 cause achromatopsia. Here, I will provide an overview on mouse models for CNG channelopathies and discuss molecular disease mechanisms. Furthermore, I will summarize recent results from preclinical gene therapy studies using adeno-associated viral vectors and discuss the efficacy and translational potential of these gene therapeutic approaches.

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IRB Status: None

Disclosures:

STYLIANOS MICHALAKIS, PHD: No financial relationships to disclose

O423

BESTROPHIN-1, AN INTRACELLULAR CL CHANNEL OF THE RETINAL PIGMENT EPITHELIUM

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Analysis of over-expressed protein described bestrophin-1 as a Ca²⁺-dependent Cl channel in the plasma membrane. However, data from mouse models could not be easily interpreted with this bestrophin-1 function. Therefore we investigated endogenously expressed bestrophin-1 in a short time cell culture model of porcine retinal pigment epithelial (RPE) cells.

Porcine RPE cells seeded at the same cell density as that in vivo formed monolayers in 24 h with transepithelial resistance higher than 400Ωcm² and maintained bestrophin-1 expression for more than 3 days. Function of bestrophin-1 was investigated using siRNA which fluorescence-tag to directly target cells with bestrophin-1 knock-down. The role of bestrophin-1 in Ca²⁺ signaling was assessed by Ca²⁺-imaging methods. RPE function was evaluated in an in vitro and in vivo phagocytosis assay.

Fluorescence labeling of polarized RPE revealed a stronger co-localization of bestrophin-1 with stim-1, a protein of intracellular Ca²⁺ stores, than with β-catenin which indicates that at least a strong proportion of bestrophin-1 localize to the membranes of cytosolic Ca²⁺ stores. Knock-down of the store-operated Ca²⁺ channel Orai-1 reduced SOCE as well as siRNA knock-down of bestrophin-1. siRNA knock-down of Orai-1 did not change the amount of released Ca²⁺ whereas it was significantly reduced when bestrophin-1 was knocked down. Phagocytosis of photoreceptor outer membranes (POS) was increased in vitro after siRNA knock-down of bestrophin-1, by Ca²⁺ channel inhibition or by blocking maxiK Ca²⁺-dependent K⁺ channels. In vivo we observed in Ca²⁺ channel Cav1.3 or in maxiK knock-out mice alterations in circadian rhythm of phagocytosis.

Endogenously expressed bestrophin-1 appeared to function as cytosolic Cl channel required to accumulate Ca²⁺ in Ca²⁺ stores. Bestrophin-1-dependent Ca²⁺ signaling regulate phagocytosis of POS by the RPE. In vivo, ion channels related to Ca²⁺ signaling regulate the circadian nature of phagocytosis. Therefore, mutation-dependent loss of bestrophin-1 function might deregulate the phagocytosis rhythm.

IRB Status: None

Disclosures:

OLAF STRAUSS, PHD: No financial relationships to disclose

JT03 – The New Concept of the Blood–Aqueous Barrier

O424

NOT ALL CLINICALLY OBSERVABLE FLARE IS PATHOLOGICAL

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Elevated aqueous humor (AH) protein levels (aka flare) have always been attributed to breakdown of the blood–aqueous barrier (BAB), regardless of cause. This was because the

original model of the BAB presumed that the plasma-derived protein (PDP) in AH was secreted with AH. In previous studies, we have shown that PDPs in AH bypass the posterior chamber (PC), reaching the anterior chamber directly from the ciliary body stroma via the iris root. Thus, the secretion of AH, and addition of its PDPs, are semi-independent pathways, allowing PDP levels to vary without invoking BAB breakdown as the explanation. Flare appearing after instillation of pilocarpine drops has always been attributed to BAB breakdown. In human studies, using MRI and intravenous gadolinium to directly observe BAB kinetics in the PC in vivo, topical 3% pilocarpine produced the expected flare but no leakage of tracer into the PC. So no BAB breakdown was seen in the ciliary body, but these methods could not confirm whether iris vascular permeability increased. To address this we completed intravascular tracer studies, using horseradish peroxidase (HRP) in rabbits. One hour after 3% pilocarpine in one eye, pupil size decreased and flare increased significantly. Microscopy demonstrated no HRP leakage from the iris vessels or ciliary epithelium. Additional animals received pilocarpine in one eye and sacrificed after 1hr, without tracer studies. Distribution of PDPs in uveal tissues was assessed by immunohistochemistry and the amount of soluble protein eluted from all irides was measured. Immunohistochemistry confirmed there were PDPs in the iris stroma. Irises from treated eyes showed significantly less elutable protein. Stronger miosis (i.e. greater change in pupil size) gave lower residual PDP levels, suggesting extrusion of stromal PDPs during strong miosis caused the flare. These studies strongly suggest that not all clinically observable flare results from breakdown of the BAB.

IRB Status: Approved

Disclosures:

THOMAS FREDDO, OD, PHD: No financial relationships to disclose

O425

THE MAGNITUDE OF THE UVEOSCLERAL INFLOW PATHWAY IN THE HUMAN EYE: EVIDENCE FROM PATIENTS WITH CORNEAL ENDOTHELIAL DYSTROPHY

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Previous results from our group strongly suggested that oxygen levels in the anterior chamber angle were regulated by fluid flow through the root of the iris from the ciliary body vasculature (Shui et al., *IOVS* 2006; Siegfried et al., *IOVS* 2010). While studying oxygen distribution in the anterior segment of patients with Fuchs' corneal endothelial dystrophy or pseudophakic bullous keratopathy (PBK) we were able to estimate the rate of fluid flow through this "uveo-scleral inflow" pathway compared to the rate of classical aqueous humor production. Patients with Fuchs' dystrophy or PBK have a deficiency in the number and function of corneal endothelial cells, resulting in swelling and opacification of the corneal stroma. We predicted that this deficiency would result in elevated oxygen

beneath the corneal endothelium, due to decreased oxygen consumption by the endothelial cells. Oxygen levels measured beneath the central cornea averaged 21 mmHg in patients with healthy corneas undergoing cataract surgery, 41 mmHg in phakic Fuchs' patients and 44 mmHg in patients with PBK. Given the flow of aqueous humor, one might expect that this oxygen would be carried to the anterior chamber angle, potentially increasing oxidative damage to the trabecular meshwork (TM) and increasing the risk of glaucoma. However, mean oxygen levels in the anterior chamber angle were unchanged, despite the more than doubling of oxygen in the central region of the anterior chamber. These results indicate that the magnitude of the uveo-scleral inflow significantly exceeds that of the classical aqueous inflow pathway. This finding implies that, throughout life, the TM is mostly exposed to lymph, not aqueous humor, and that damage to the TM may result more from substances in the blood than in the aqueous humor.

IRB Status: Approved

Disclosures:

DAVID BEEBE, PHD: No financial relationships to disclose

O426

BETWEEN THE BLOOD AND AQUEOUS HUMOR: DEFINING A THIRD COMPARTMENT IN BLOOD-AQUEOUS BARRIER KINETICS

JAY MCLAREN

MAYO CLINIC

Movement of substances across the blood-aqueous barrier has been considered a one-step process. However, after systemic administration, fluorescein enters the anterior chamber by a slower kinetic than this simple two-compartment model (cornea and anterior chamber) predicts. The slower kinetic is consistent with a third compartment that substances must pass through to enter the anterior chamber.

To test this three-compartment model, fluorescein was measured in the anterior chamber in 6 human participants after injection of fluorescein intravenously. The transfer coefficient between blood and aqueous humor in the two-compartment model, and transfer coefficients between blood and a third compartment and between this compartment and aqueous humor in the three-compartment model, were adjusted until each model best matched concentrations measured. The goodness of fit of each model was examined by calculating the sum of squared differences between the predicted and measured concentrations. Both models were also fitted to measurements from 12 rabbits during the first 60 minutes after intravenous fluorescein injection.

In both humans and rabbits, the best-fitted three-compartment model matched the appearance of fluorescein in the anterior chamber better than did the best-fitted two-compartment model. In humans, the sums of squared differences were 22 ± 28 and 306 ± 150 for the three- and two-compartment models respectively (mean \pm SD, $p=0.008$, $n=6$). In rabbits, the sums of squared differences were 71 ± 65 and 888 ± 690 ($p=0.001$, $n=12$) for three- and two-compartment models respectively.

The better fit of the three-compartment model suggests that substances cross the blood–aqueous barrier in two steps, the first from blood to a third compartment and then from this compartment to aqueous humor. The iris stroma is the most likely anatomic structure for this third compartment.

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IRB Status: Approved

Disclosures:

JAY MCLAREN, PHD: No financial relationships to disclose

0427

FLUID FLOW FROM CILIARY CAPILLARIES TO ANTERIOR CHAMBER: A PARALLEL PATHWAY CONTRIBUTING TO AQUEOUS HUMOR DYNAMICS

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There is accumulating experimental evidence that solutes and fluid can be transferred between the ciliary body stroma (CBS) and the anterior chamber (AC) via the anterior surface of the iris, bypassing the ciliary epithelium (CE). Work by T. Freddo (*Exp Eye Res.* 2001) indisputably shows spontaneous diffusion

of proteins from the CBS to the AC angle in the absence of hydrostatic pressure difference (HPD). The importance of this parallel pathway has been disregarded due to the lack of a substantial HPD acting as a driving force between the two compartments (Bill, A. *Physiol Rev.* 1975). However, we reasoned that if an HPD is elicited between these compartments, this could induce a fluid flow from the CBS to the AC. We accomplished this in two ways, by reducing pressure in the AC (a) and by increasing pressure in the CBS (b): a) Paracentesis of 60, 120 and 300 μ L was done in sheep. The time required to restore the control IOP was measured and an average flow of up to 6 μ L/min was calculated. This was larger than what transport across the CE could support and, thus, we concluded that it represented a plasma-like substance flowing directly from the ciliary capillaries to the AC. b) 100 mg of Sildenafil was given by mouth to sheep. We have shown that this increased the IOP in sheep and humans. Thus, it increases the AH turnover to values that cannot be supported by the CE transport system. We conclude that the parallel pathway is operative in certain situations in which pressure is decreased in the AC and that normal pressure can be restored with sildenafil. This could be tested as an adjunct in surgical procedures to restore AC volume. It may also have utility in cases of ocular hypotony.

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IRB Status: None

Disclosures:

OSCAR CANDIA, MD: No financial relationships to disclose



ABSTRACTS - POSTERS



International Society
for Eye Research

Anterior Segment

Viewing: 10:00 – 10:30
12:00 – 13:00

Session with Authors: 15:00 – 16:30

P101

RNA SEQUENCING-BASED COMPARATIVE TRANSCRIPTOME ANALYSIS OF BALB/C AND C57BL/6 MOUSE CORNEAS OF NEOVASCULARIZATION

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Corneal neovascularization (lymphangiogenesis and angiogenesis) accompanies many diseases and is critically involved in immune responses and transplant rejection. We have shown that this process varies significantly between different mouse strains, such as BALB/c and C57BL/6, which are most commonly used in corneal neovascularization and transplantation research. To better understand the molecular mechanisms behind this phenomenon, we used novel RNA sequencing technique and investigated RNA expression profiles of the corneas of these strains under both normal and suture-induced vascularized conditions. Three biological replicates were applied in each group for statistical analysis. Our data showed that a total of 1372 and 374 genes were differentially expressed in sutured versus normal corneas of BALB/c and C57BL/6 mice, respectively. Further analysis revealed that 305 genes were differentially expressed between normal corneas of BALB/c and C57BL/6 mice, and 630 genes were significantly altered between sutured corneas of these two strains. A majority of these altered genes from sutured corneas were clustered into categories of immune response, inflammatory response, angiogenesis, signal transduction, and extracellular matrix, and those genes of the normal corneas were mainly separated into groups of extracellular region, glycoprotein, signal, immune response and defense response. Further downstream experiments are undertaken to confirm the significant findings and to isolate specific genes that are critically involved in processes of lymphangiogenesis and angiogenesis. To our knowledge, this is the first *RNA sequencing-based transcriptome* study on differential gene expression induced by corneal neovascularization.

IRB Status: None

Disclosures:

GUANGYU LI: No financial relationships to disclose

P102

INFLUENCE OF MORPHOLOGIC ALTERATIONS OF DESCMET'S MEMBRANE ON THE CORNEAL EDEMA IN FUCHS' ENDOTHELIAL DYSTROPHY

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Fuchs endothelial corneal dystrophy (FED) is characterized by morphologic alterations of the endothelium–Descemet's membrane (EDM), including endothelial cell loss, EDM thickening and transformation of the extracellular matrix. This results in corneal edema and loss of visual acuity. While barrier and pump dysfunctions are being discussed as causative for corneal edema in FED, definite mechanisms remain insufficiently understood. The aim of this study was to investigate the influence of structural alterations within the EDM on the central corneal thickness (CCT), which is a measure for corneal edema. Thirty-one eyes of 21 patients underwent Descemet's membrane endothelial keratoplasty for FED. Intraoperatively obtained recipients' EDM were investigated histologically and immunohistochemically. Following, morphologic parameters were correlated to CCT. The mean CCT measured $660 \pm 53 \mu\text{m}$. Histologically, the EDM thickness measured $16.2 \pm 3.6 \mu\text{m}$ and the anterior banded layer (ABL) thickness was $3.0 \pm 0.8 \mu\text{m}$. The CCT did not correlate with the EDM thickness or the presence and size of endothelial guttae. A significant correlation was found between CCT and ABL thickness ($r = 0.464$, $p = 0.020$). This correlation was even increased, taking interindividual variations into account ($r = 0.692$, $p = 0.006$). Immunohistochemically, matrix proteins were rarified within the ABL. Fibronectin and cytokeratin were found in 26% and 16% of the cases, respectively. However, CCT or ABL thickness did not correlate with a loss of matrix proteins. Our findings revealed that ABL thickness is positively associated with CCT in FED. Thereby, barrier dysfunctions might primarily cause corneal edema in those patients. FED-associated genetic mutations affect the COL8A2 gene, which encodes the $\alpha 2$ chain of type VIII collagen, an essential ABL component. Thus, malformations of the ABL are likely to be associated to typical FED manifestations such as corneal edema. Therefore, phenotype–genotype analyses are intended to reveal further distinctions.

IRB Status: Approved

Disclosures:

TOBIAS BROCKMANN: No financial relationships to disclose

P103

EVALUATION OF A NOVEL ARTIFICIAL TEAR IN THE PREVENTION AND TREATMENT OF DRY EYE IN AN ANIMAL MODEL

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Purpose: To evaluate the efficacy of a novel product that combines carboxymethylcellulose (CMC) and Hyaluronic acid (HA) on the prevention and treatment of dry eye in a murine model.

Methods: Dry eye was induced in mice (C57BL/6) using an intelligently controlled environmental system (ICES). Three products containing CMC + HA (Optive Fusion, OF), CMC only (Refresh Tears, RT) and HA only (Hycosan, HS) respectively were tested and compared with PBS, and ICES with no intervention (NT). Two schedules were employed to investigate the prevention and treatment effects respectively: prevention was assessed by concurrent drop administration with ICES; treatment was assessed by drop administration following development of dry eye. The study variables included: corneal fluorescein staining, corneal epithelial tight junction (occludin) and cornification (small proline-rich protein, SPRR-2), apoptosis (caspase-3 and TUNEL assay), inflammatory mediators (including IL-1 β , IL-6, IL-17, IL-23 and TNF- α), and conjunctival goblet cell counting.

Results: All three treatment groups showed significantly better effects than PBS and NT groups in all measures ($p < 0.05$). Compared with RT and HS, OF showed significantly lower corneal fluorescein staining ($p < 0.01$), higher goblet cell density ($p < 0.05$), and lower apoptosis ($p < 0.05$). Corneal epithelial occludin staining displayed a more homogenous distribution, and SPRR-2 labeling revealed a lower expression in the OF group compared with the RT and HS groups. The prevention and treatment regimens showed similar results for these data. However, for inflammatory mediators, the results were variable among the treatments and regimens.

Conclusions: The novel product showed good efficacy in preventing and treating the environmentally-induced dry eye in both clinical evaluation and tissue measurements. Differential expression patterns of the cytokines suggested varying functions of each in the development of ocular surface inflammation.

IRB Status: None

Disclosures:

WEI CHEN: Contracted Research relationship with Allergan, Inc.

P104

STABILITY OF A NOVEL BACTERIAL LIPASE INHIBITOR IN THE PRESENCE OF BACTERIA AND BASAL TEARS

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Recent findings signal a role for the ocular microbiome in ocular surface health and in the escalation of common surface disorders. Changes in commensal bacterial profiles can result in altered expression of bacterial exoproducts including lipases which might alter the tear lipid spectrum and contribute to dry eye disease. Glycerol monolaurate (GML) inhibits lipase production by Gram positive bacteria without affecting bacterial growth. However, human and staphylococcal lipases can also degrade GML. We sought to assess lipase production by common ocular staphylococcal spp. isolates and assess GML lipase inhibition in the presence of bacterial and human tear lipases.

Staphylococcus epidermidis (20 isolates) and *Staphylococcus aureus* (20 isolates) were cultured in 10 ml tryptic soy broth at 37°C. After overnight incubation lipase activity of culture supernatants was quantified using a commercial lipase assay kit and normalized against bacterial growth. Bonferroni multiple comparison test in ANOVA was applied to compare average lipase production. To determine GML activity in the presence of bacterial and tear lipases, GML (80 μ g/ml) was added to centrifuged suspensions of overnight cultures of *S. aureus* 020 or basal tears and incubated up to 4 hours. Nascent lipase production in the presence of this 'conditioned' GML was measured by the addition of *S. aureus* (103ml-1), followed by incubation and quantification as above. Lipase production by *S. aureus* strains (2.37 ± 0.54 log units) was higher than *S. epidermidis* strains (2.02 ± 0.37 log U, $p = 0.038$). In the presence of bacterial lipases or tears, GML was active for up to 4 hours, inhibiting bacterial lipase production without affecting bacterial cell number relative to control. In conclusion all ocular staphylococcal spp. isolates exhibited lipase activity. GML lipase inhibition was stable in the presence of *S. aureus* 020 and tear lipases, and inhibited nascent bacterial lipase production.

IRB Status: None

Disclosures:

JUDITH FLANAGAN: No financial relationships to disclose

P105

PDGF-BB EFFECTS DURING HUMAN CORNEAL STROMA WOUND REPAIR IN VITRO

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Understanding of the production and function of growth factors in ophthalmic tissues as the cornea, will lead to improve insights into tissue maintenance and modulation of the wound healing process, where growth factors play a significant role in directing cellular behavior and ultimately to more effective treatment regimens. The aim of this work was to determine the effect of PDGF-BB during corneal stromal wound repair in vitro. The effect of PDGF-BB on wound closure time, proliferation, migration, differentiation and extracellular matrix (ECM) synthesis was evaluated in human corneal fibroblasts (HCFs) cultures. After making wounds (700 μ m in width), HCFs were cultured in Dulbecco's modified Eagle's medium (DMEM/F12) supplemented with PDGF-BB or without it (control), and 5-bromo-2'-deoxyuridine as a cell proliferation marker. Cultures were analyzed from day 1 to 15. In control wounds we observed a slow closure process, and an increase in proliferation and differentiation to myofibroblasts over time. In PDGF-BB treated wounds the closure was faster than in the control, the proliferation process was higher than in the control and it

took place at earlier times. Differentiation to myofibroblasts was slow and gradual. HCFs treated with PDGF-BB appear like typical fibroblasts with fusiform spindle shaped but showed more spindle shaped morphology than that observed in control. The mRNA expression of perlecan, syndecan-4, focal adhesion kinase, and $\alpha 5 \beta 1$ integrin (all of them present during the normal stromal wound healing in vivo) was up-regulated in PDGF-BB. On the other hand, the mRNA expression of collagen type III (a scarring and fibrosis marker) was undetected in both control and PDGF-BB treated HCFs. The results showed that the application of PDGF-BB accelerated the wound healing process, avoiding the excessive myofibroblast differentiation and synthesizing a repair ECM without fibrosis.

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IRB Status: International

Disclosures:

PATRICIA GALLEG0: No financial relationships to disclose

P106

A NOVEL PRESSED POROUS SILICON-POLYCAPROLACTONE COMPOSITE AS A DUAL-PURPOSE OPHTHALMIC IMPLANT

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FLINDERS UNIVERSITY OF SOUTH AUSTRALIA¹; FLINDERS UNIVERSITY, DEPARTMENT OF ANATOMICAL PATHOLOGY²; TEXAS CHRISTIAN UNIVERSITY, DEPARTMENT OF CHEMISTRY³; FLINDERS UNIVERSITY OF SOUTH AUSTRALIA, DEPARTMENT OF OPHTHALMOLOGY⁴; UNIVERSITY OF SOUTH AUSTRALIA, MAWSON INSTITUTE⁵

Dysfunction of the adult corneal stem cells can lead to painful disease of the ocular surface. Allogeneic limbal tissue transplants have a poor prognosis. *Ex vivo* expanded stem cells must be transferred to the eye on a scaffold. We hypothesised that composite materials of nanostructured porous silicon (pSi) microparticles (MPs) and polycaprolactone (PCL) could be used as a scaffold to transfer cells and drugs to the eye. In this study, we fabricated a non-woven polycaprolactone fabric and pressed porous silicon microparticles on to the fibers. Human lens epithelial cells attached to, and grew upon, both the fibres and pSi particles of the composite. After 6 hours, cells on or in the vicinity of fluorescein diacetate (FDA) loaded porous silicon microparticles exhibited intracellular fluorescence, indicative of transfer of FDA into viable cells from the pSi, with subsequent cleavage to fluorescein. Human lens epithelial cells showed enhanced growth on fetal bovine serum coated, rather than uncoated materials. BALBc/3T3 cells showed significantly more proliferation at 48 hours when seeded on materials loaded with a mixture of protein growth factors containing epidermal growth factor, insulin and transferrin, than on unloaded composites. No proliferation of BALBc/3T3 cells was observed on polymer without pSi, indicating that the biologics preferentially loaded into the pSi MPs. Implantation of the composites under the conjunctiva in rats did not cause

significant inflammation, neovascularization, nor elicited a lymphocyte-mediated immune response. A macrophage and giant cell-mediated foreign-body response, similar to that mounted against sutures, was observed. This novel pressed pSi-PCL composite material has potential for delivery of small drugs, can be loaded with biologics that are released from the material in active form, can support the growth of mammalian cells, and could be of use as a support for an artificial stem cell niche in ocular surface disease.

IRB Status: International

Disclosures:

YAZAD IRANI: No financial relationships to disclose

P107

PREPARATION OF OPHTHALMIC FORMULATIONS CONTAINING ITS NANOPARTICLES

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KINKI UNIVERSITY, FACULTY OF PHARMACY¹; KINKI UNIVERSITY, FACULTY OF MEDICINE²

[Background and Aims] The ophthalmic application of drugs is the primary route of administration for the treatment of various eye diseases, and is well-accepted by patients; however, there is a need for frequent instillation of concentrated solutions to obtain the desired therapeutic effect in both the anterior and posterior hemispheres of the eye. Therefore, it is very important to increase the effectiveness of drugs by enhancing their bioavailability. In this study, we prepared ophthalmic formulations containing tranilast (TL) nanoparticles and investigated their usefulness in the ophthalmologic field by evaluating corneal toxicity and permeability.

[Methods] The TL was added in the solution containing 0.005% benzalkonium chloride (BAC), 0.5% D-mannitol, and 5% 2-hydroxypropyl-beta-cyclodextrin, and the dispersions containing TL nanoparticles (TLnano) was prepared by using Bead Smash 12. The antimicrobial activity was tested by *Escherichia coli* (ATCC 8739). The transcorneal penetration of ophthalmic dispersions containing 0.5% TL or commercially available TL eye drops was examined by rabbit, and the immortalized human corneal epithelial cell line was used for evaluation of cytotoxicity.

[Results] TL nanoparticles were prepared using zirconia beads and Bead Smash 12, which allowed the preparation of high quality dispersions containing 0.5% TL nanoparticles (particle size, 34 nm). The dispersions containing TL nanoparticles are tolerated better by human corneal epithelium cells than a commercially available TL preparation (RIZABEN® eye drops). In addition, the addition of TL nanoparticles to the dispersions does not affect the antimicrobial activity of BAC against *Escherichia coli*, and the corneal penetration of TL from dispersions containing TL nanoparticles was significantly higher than in the case of the commercially available TL eye drops.

[Conclusions] It is possible that dispersions containing TL nanoparticles will show increased effectiveness in treating

ocular inflammation, and an ocular drug delivery system using drug nanoparticles may expand their usage for therapy in the ophthalmologic field.

IRB Status: International

Disclosures:

YOSHIMASA ITO: No financial relationships to disclose

P108

OCULAR PHARMACOKINETICS COMPARISON BETWEEN PATADAY® VERSUS 0.77% NEWLY DEVELOPED OLOPATADINE TO MALE NZW RABBITS

GANESH IYER, Jaime Yanez, Scott Womble, James Chastain

ALCON RESEARCH LTD, A NOVARTIS COMPANY

To compare uptake and distribution of PATADAY® (0.2% olopatadine) versus the newly developed 0.77% olopatadine HCl (0.7% free base) ophthalmic formulation following a single (~30 µL), bilateral topical ocular dose in male New Zealand White (NZW) rabbits. The 0.77% olopatadine formulation differs from PATADAY (olopatadine ophthalmic solution, 0.2%) in the concentration of olopatadine and the inclusion of 3 excipients to enhance the solubility of olopatadine at a neutral pH. Each animal received a single 30 µL bilateral topical ocular dose (PATADAY or 0.77% olopatadine) to the right (OD) eye followed by the left (OS) eye for a total dose of 60 µL. Following administration, blood samples of plasma were collected at pre-specified time points (0.5, 1, 2, 4, 6, 8, 12 and 24 hours) prior to euthanasia. Following homogenization of the ocular tissue samples (except aqueous humor), olopatadine concentrations in cornea, bulbar conjunctiva, choroids, iris-ciliary body (ICB), whole lens (with lens included) and retina were assayed using a validated liquid chromatography tandem mass spectrometry analytical method (LC/MS/MS). Olopatadine was absorbed into the eye and reached maximal levels (C_{max}) within 30 minutes to 2 hours (T_{max}) in ocular tissues. The greatest differences between the PATADAY versus 0.77% olopatadine were associated with the overall duration of exposures. The mean C_{max} estimates in aqueous humor, choroids, ICB and lens increased with increasing concentrations of olopatadine. Olopatadine concentrations were observed up to 24 hours following 0.77% olopatadine versus 4 hours to 12 hours after PATADAY administration. The 0.77% olopatadine formulation was able to overcome the aqueous solubility at a neutral pH, a limiting factor in formulating olopatadine containing eyedrops. The newly developed ophthalmic formulation allowed olopatadine to remain dissolved in a stable solution at a concentration of 0.77% resulting in a greater increase in ocular bioavailability as compared to 0.2% PATADAY.

IRB Status: None

Disclosures:

GANESH IYER: Employee relationship with Alcon Research Ltd, a Novartis Company

P109

KERATOCONUS: ROLE OF THE TGF-β SIGNALING PATHWAY

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Keratoconus (KC) is an abnormal cone-shaped protrusion of the cornea. It affects 1:500/1:2000 people worldwide but there are significant difficulties with differential diagnosis that cause uncertainty as to its prevalence. In the absence of any animal models, we have recently developed a 3D in vitro model which can be used for KC in vitro studies and the identification of new bio markers. We are focused on identifying cellular mechanisms and pathways that may help understand the disease better and provide new clinically relevant advancements. Transforming growth factor-β (TGF-β) has been identified as key player in the modulation of the KC extracellular matrix (ECM). Here we attempt to identify similarities and differences in the fibrotic markers, receptors, and down-stream pathways when human keratoconus cells (HKCs) are stimulated by the 3 TGF-β isoforms: TGF-β1 (T1), -β2 (T2), and -β3 (T3). Human corneal fibroblasts (HCFs) served as Controls. We investigated two different systems: a) 2D cultures and b) 3D constructs. HCFs and HKCs were grown in 10% human serum and stimulated by a stable form of ascorbic acid. We identified several significant differences upon TGF-β stimulation. Both in HKCs and HCFs, Smooth muscle actin (SMA) and Type III collagen, both fibrotic indicators, were significantly down regulated upon T3 stimulation. Surprisingly neither of them was up regulated upon T1 stimulation, in HKCs. TGFβ-Receptors were also analyzed (TGFB1, -R2, and -R3). TGFB1 was up regulated with T3 while TGFB2 and TGFB3 were down regulated. No significant differences were found for TGFB1 and TGFB2 between Controls and T1 or T2. TGFB3 however, was up regulated upon T1 stimulation. Minimum regulation was noticed between conditions for SMAD-2, -3, and -4. In summary, current knowledge and the present study suggest that KC pathogenesis could be related to activation of the TGFβ pathway; however, its involvement requires further investigation.

IRB Status: International

Disclosures:

DIMITRIOS KARAMICHOS: No financial relationships to disclose

P110

HUMAN CORNEAL STROMA WOUND REPAIR IN VITRO AFTER BFGF TREATMENT

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After an injury, the keratocytes “activated” phenotypes help in the regeneration of a normal stromal matrix or can become scars. These phenotypes are dependent on specific environmental signals such as growth factors. Comprehension of the role played by each growth factor, will lead to improving our knowledge into tissue maintenance and wound healing modulation. The aim of this work was to determine the effect of bFGF during stromal wound repair. The bFGF effects on wound closure time, proliferation, migration, differentiation and extracellular matrix synthesis were evaluated in human corneal fibroblasts (HCFs) cultures. After making wounds (700 µm in width), HCFs were cultured in Dulbecco’s modified Eagle’s medium (DMEM/F12) supplemented with bFGF or without it (control), and 5-bromo-2’-deoxyuridine as a cell proliferation marker. Cultures were analyzed from day 1 to 15. Closure time followed a similar pattern in both groups, but in bFGF cultures the closure was faster than in control. Proliferation was higher in bFGF than control over time and this process was due to an increase of BrdU positive cells in the wound area. Myofibroblast differentiation was slow and gradual in controls; on the contrary this differentiation was inhibited in bFGF treated wounds. No changes in morphology were observed. The mRNA expression of perlecan and syndecan-4 was up-regulated after bFGF treatment. On the other hand, the expression levels of focal adhesion kinase and $\alpha 5 \beta 1$ integrin were similar in both groups. Finally, the expression of collagen type III was undetected in both groups. The results showed that the bFGF treatment accelerated the healing process, by an increase in cellular proliferation in the wound, inhibiting myofibroblast differentiation and showing low levels of migration. These results mimic the initial hypercellular phase that takes place in vivo during corneal wound healing.

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IRB Status: International

Disclosures:

CARMEN MARTINEZ-GARCIA: No financial relationships to disclose

P111

CONDITIONAL DELETION OF AP-2 β IN NEURAL CREST CELL POPULATIONS RESULTS IN DYSGENESIS OF STRUCTURES IN THE ANTERIOR SEGMENT OF THE EYE

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Fate-mapping of the vertebrate eye has confirmed that the neural crest cell (NCC) population contributes to the formation of many structures of the anterior segment of the eye including the corneal endothelium and stroma, iridocorneal angle and ciliary body. However, the mechanisms responsible for migration and differentiation of this cell population have yet to be fully elucidated. Members of the Activating Protein-2 (AP-2) family of transcription factors have been shown to be

expressed in the NCC population in the eye. Studies from our laboratory have outlined the importance of this transcription factor family during ocular genesis. In the current study we investigate the contribution of AP-2 β gene expression in the NCC population during development of the anterior segment of the eye. Utilizing the Wnt-1 Cre mouse line, a mouse mutant was generated with conditional deletion of AP-2 β in the NCC population of the eye. The eyes were examined at post-natal stages via histological and immunofluorescent techniques. The AP-2 β NCC null mutants displayed both morphological and cellular defects in a number of structures of the anterior segment of the eye. These include a disrupted iridocorneal angle with the iris adhering to the corneal stroma, a disorganized and hypercellular corneal stroma, and reduced stratification of the corneal epithelium. Immunohistochemistry confirmed little to no N-cadherin staining of the corneal endothelium, in addition to confirmed presence of subcapsular cataracts via α -smooth actin staining in some samples. Thus far our findings demonstrate the importance of AP-2 β gene expression in neural crest cells in the proper development of multiple structures of the anterior segment of the eye.

IRB Status: None

Disclosures:

VANESSA MARTINO: No financial relationships to disclose

P112

THE HUMAN TEAR LIPIDOME: DAY-TO-DAY VARIATION AND THE DIFFERENCES BETWEEN INDIVIDUALS

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Knowledge of the complete human tear film lipidome is vital in understanding the pathology of dry eye syndrome. Lam et al. (*PLoS ONE* 2011) have shown that dry eye is associated with a reduction in (O-acyl)-omega-hydroxy fatty acids (OAHFAs). Nevertheless, there is a paucity of data describing if or how our tear film lipidome differs between individuals and if it remains constant from day-to-day. This project utilized highly sensitive mass spectrometry-based analytical protocols to answer these questions. Basal tears were collected from 4 individuals (20-35 years of age) with a glass capillary at the lower lid margin without stimulating reflex tears. Each individual was sampled at approximately the same time by the same investigator on 3 consecutive days. The choice of either left or right eye was randomized and kept constant for each participant throughout the study. Lipids were extracted in methyl tert-butyl ether and analyzed using chip-based nano-electrospray ionization mass spectrometry. Targeted precursor ion and neutral loss scans were used to identify and quantify the molecular composition of OAHFAs, phosphatidylcholines, phosphatidylethanolamines, phosphatidylserines, sphingomyelins, wax esters, cholesteryl esters and triacylglycerols. A total of 236 individual lipid species were identified and quantified in human tears. These lipids were found in 9 lipid classes

including cholesteryl esters, wax esters, OAHFAs, triacylglycerols, phosphatidylcholine, lysophosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and sphingomyelin. Wax esters (43±4%) and cholesteryl esters (39±3%) were the dominant lipid classes while total phospholipids were also a significant contributor to the tear lipidome, they were found to be highly variable between individuals (12±7%). Principle component analysis was able to clearly separate individuals based on their tear lipid profile. No significant daily variation within an individual's tear lipid profile was observed. These data indicate that each person's tear lipidome is unique and that it remains constant from day-to-day.

IRB Status: International

Disclosures:

TODD MITCHELL: No financial relationships to disclose

P113

EFFECTS OF LOSS OF TRPM2 ON THE INFLAMMATION AND SCARRING AFTER AN ALKALI-BURNED CORNEA IN MICE

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Purpose: To determine if transient receptor potential melastatin 2 (TRPM2) gene ablation affects inflammation and scarring severity in a healing, alkali-burned, mouse cornea. TRPM2 is one of the TRP family cation channels and is reportedly important in inflammatory processes.

Methods: 1) Three μ L of 1 N NaOH were applied under general anesthesia to the right eye of 6-8 week old TRPM2-null (KO) or wild-type (WT) mice to produce an ocular surface alkali burn. The eyes were processed for histology, immunohistochemistry or real time RT-PCR. 2) Ocular fibroblasts from mouse eyes were used to study the role of TRPM2 for pro-inflammatory gene expression. 3) The role of bone marrow-derived inflammatory cells in the KO phenotype of healing was examined in WT or KO mice that had received bone marrow transplantation (BMT) from either genotype of mice.

Results: 1) Stroma of the KO healing corneas was less opaque as compared with those of WT mice at 5 to 20 days post-alkali burn. Immunohistochemical and real time RT-PCR examinations show less appearance of myofibroblasts and less invasion of both leukocytes and macrophages in the cornea of KO mice after alkali burn. 2) TRPM2 gene ablation suppressed mRNA expression of IL-6, MCP-1 and TGF β 1 in cultured KO ocular fibroblasts. Exogenous TGF β 1 up-regulated Collagen1a1 mRNA expression more prominently in WT cells than in KO cells. 3) The KO bone marrow-derived cells, but not the KO corneal tissue resident cells, are responsible for the KO-type wound healing.

Conclusions: The loss of TRPM2 improved the corneal wound healing response against an alkali exposure with suppression of inflammation and scarring. The presence of TRPM2 gene in bone marrow-derived cells are responsible for mediating inflammatory responses during the healing of corneal alkali burn.

IRB Status: None

Disclosures:

YUKA OKADA: No financial relationships to disclose

P114

INHIBITION OF LYMPHANGIOGENESIS AND HEMANGIOGENESIS IN CORNEAL INFLAMMATION BY SUBCONJUNCTIVAL PROX1 siRNA INJECTION IN RATS

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Prospero homeobox 1 (Prox1) siRNA is a small interfering RNA that is designed to specifically bind Prox1 mRNA. We determined whether Prox1 siRNA inhibits lymphangiogenesis and hemangiogenesis after acute corneal inflammation. Three Prox1 siRNAs were synthesized and tested for their effect on Prox1 mRNA expression in human dermal lymphatic endothelial cells (HDLECs) *in vitro*. Then, the *in vivo* effects of Prox1 siRNA were assessed in alkali burn-induced inflammatory corneal neovascularization of rats. Prox1 siRNA was administered via subconjunctival injection. Corneal flat mounts were stained with lymphatic vessel endothelial hyaluronan receptor (LYVE)-1 to reveal lymphatic vessels. Lymphangiogenesis and hemangiogenesis were analyzed morphometrically using Image J software. Corneal inflammatory cell infiltration was evaluated by immunostaining for F4/80 and CD45. We found that Prox1-1 treatment decreased Prox1 mRNA expression in cultured HDLECs. Subconjunctival injection of Prox1-1 significantly inhibited alkali burn-induced lymphangiogenesis ($p = 0.03$) and hemangiogenesis ($p = 0.005$) in the cornea compared to those of scrambled siRNA (negative control). This inhibition was comparable to that induced by bevacizumab (positive control). Prox1 knockdown by Prox1-1 also inhibited macrophage and leukocyte infiltration into the cornea. These results indicate that Prox1 siRNA is a strong inhibitor of inflammatory corneal lymphangiogenesis and hemangiogenesis *in vivo*. Prox1 siRNA may be useful in preventing immune rejection after penetrating keratoplasty by suppressing lymphangiogenesis.

IRB Status: Approved

Disclosures:

CHANG RAE RHO: No financial relationships to disclose

P115

HISTOLOGICAL EXAMINATION OF THE CORNEAL FILAMENT IN FILAMENTARY KERATITIS

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Filamentary keratitis is related to severe dry eye and various kinds of ocular surface diseases. The current number of re-

ported studies regarding filaments is limited, and there are no histological reports on how the filament continues to the corneal epithelium. We histologically examined 5 cases of corneal filaments observed in 48 cases of cat dry-eye model induced by resection of the lacrimal gland. Frozen cornea sections containing the filament were subjected to hematoxylin and eosin staining or indirect immunostaining analysis. The other cornea section containing the filament was fixed with glutaraldehyde and osmium tetroxide, and was examined using scanning and transmission electron microscopy. Immunostaining showed that the attached area of filament was positive for corneal epithelium marker, cytokeratin (CK) 12. The head area of the filament was comprised of CK12- and CK13 (conjunctival epithelium maker)-positive cells. The filament stained positively for mucins, MUC5AC and MUC16. Electron microscopy showed that desquamated epithelial cells and inflammatory cells were contained in the homogeneous material, and that the filament continued with corneal epithelial cells to the corneal surface. The filament was composed of corneal and conjunctival epithelium, inflammatory cells, and mucins. Our findings show that the attached area of the filament is composed of corneal epithelial cells.

IRB Status: None

Disclosures:

HIDETOSHI TANIOKA: No financial relationships to disclose

P116

DISTRIBUTION OF GLYCOSYLATED PROTEINS ON THE SURFACE OF CORNEAL EPITHELIAL CELL CULTURES

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UNIVERSITY OF CALIFORNIA DAVIS, SURGICAL AND RADIOLOGICAL SCIENCES¹; UNIVERSITY OF WISCONSIN MADISON, CHEMICAL AND BIOLOGICAL ENGINEERING²; UNIVERSITY OF CALIFORNIA, DAVIS, WILLIAM R. PRITCHARD VETERINARY MEDICAL TEACHING HOSPITAL³

Dry eye diseases (DED), a heterogeneous group of ocular surface disorders, are characterized by poor tear film stability. Factors that have been implicated include 1) impaired production of the aqueous component of tears, 2) enhanced evaporation and/or 3) reduced wettability of the epithelial surface. The ocular epithelium is covered by a thick glycocalyx (composed predominantly of membrane-associated mucins and other glycosylated proteins), which is traditionally thought to influence the wettability of the ocular surface. We devised an assay using fluorescently labeled microbeads functionalized with either Galectin 3 (GAL3; β -galactosidase binding) or mucin-1 monoclonal antibody (anti-MUC1), to evaluate the spatial distribution of glycosylated proteins and mucins on the apical surface of the corneal epithelium. Human hTERT immortalized corneal epithelial cells (hTCEpi) were cultured in serum-free growth medium (GM; Epilife® supplemented with Epilife defined growth supplement) to 100% confluence, after which the GM was replaced with stratification medium (SM; DMEM/F12 supplemented with 10 ng/mL EGF and 10% FBS) and cultured for up to 7 days to induce mucin expression. Cells

cultured in GM or SM for various durations were exposed to functionalized microbeads in PBS for 30 minutes, rinsed thoroughly and their surface localization determined by epifluorescence microscopy. β -galactosidases and MUC1 were observed to be evenly distributed on hTCEpi cells surface when cultured in GM. However, in SM, the glycosylated proteins were spatially distributed in patches, suggesting chemical heterogeneity on the cell surface, and resembled the reported distribution of dark and light cells across the corneal epithelium as imaged by scanning electron microscopy. These findings suggest that with increasing differentiation, chemical heterogeneity of the ocular surface is increased and this heterogeneity may play a role in tear film stability.

IRB Status: None

Disclosures:

BERNARDO YANEZ SOTO: No financial relationships to disclose

P117

THREE NOVEL MUTATIONS, P24R, G31R AND G463R IN COL8A2 GENE OF KOREAN PATIENTS WITH FUCHS' CORNEA DYSTROPHY

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To investigate the genetic basis of the Fuchs' corneal dystrophy (FCD), we screened mutations of collagen type VIII alpha 2 (COL8A2) gene located on 1p34.3-p32. Genomic DNA was extracted from blood samples of eight families included 18 affected patients and 34 individuals with FCD visited the Department of Ophthalmology at the Catholic University Medical Center. Polymerase chain reaction and direct sequencing were used to screen genetic variations in COL8A2. Control individuals (n=70) were selected from general population without Fuchs' corneal dystrophy. Screening of COL8A2 gene revealed four nonsynonymous mutations, four synonymous and two polymorphisms in Korean FCD patients. Among them, we detected five novel mutations; three nonsynonymous mutations, P24R in one family and three individual patients, G31R in one individual and G463R two individuals, and two synonymous mutations, L8L in six individuals and I464I in one individual, respectively. And also we detected five reported variations. In 11 patients of three families, we found a heterozygous two base-pair transitions from CA to GT in exon 2, resulting in a substitution of Valine by Glutamine (Q455V). In three families and 30 individual patients, we found a heterozygous single base pair transition from C to T (ACG->ATG) in the second nucleotide position of codon 502 (T502M). And we found heterozygous for R155Q in one family and 15 individual patients. Two mutations, R155Q and T502M, and were also found in unaffected individuals. In this study, we identified four nonsynonymous mutations, which included a reported mutation Q455V, and three novel mutations, P24R, G31R and G463R, in COL8A2 of Korean patients with Fuchs'

corneal dystrophy. Our result provides that these mutations are a genetic susceptibility factor for the development of Korean patients with Fuchs' corneal dystrophy.

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Disclosures:

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P118

COMPARISON OF ENDOTOXIN-INDUCED UVEITIS MODEL AND EXPERIMENTAL AUTOIMMUNE UVEITIS MODEL IN LEWIS RATS FOR DRUG SCREENING

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TOXIKON CORPORATION

The study is to compare Endotoxin-Induced Uveitis (EIU) and Experimental Autoimmune Uveitis (EAU) Models in Lewis Rats. The EIU model was induced by endotoxin named lipopolysaccharides (LPS) through footpad injection on Day 0 in 49 Lewis rats included: Saline; dexamethasone (Dex) + low dose LPS; Dex + high dose LPS; low dose LPS; and high dose LPS. The EAU model induced by a subcutaneous injection of peptide R16 of bovine interphotoreceptor Retinoid Binding Protein (IRBP) on Day 1 in 30 Lewis rats included: Saline; Dex + IRBP and IRBP. Animals were sacrificed on Day 2/3 (EIU), and on 21 (EAU). In EIU, inflammation of eyes in Dex. + LLPS, Dex + HLPS, LLPS and HLPS groups was scored significantly higher than the Saline group ($P < 0.05$). The inflammation scores in LLPS and HLPS groups were significantly more severe than scores in Dex + LLPS, Dex + HLPS groups ($P < 0.05$). The inflammation presented a peak at 48 hours. The inflammatory cells in eye tissues in Dex + LLPS, Dex + HLPS, LLPS and HLPS groups were significantly increased when compared to the Saline group ($P < 0.05$). Concentration levels of ICAM-1, IL-6, MCP-1 and TNF- α in retina increased in various extents in Dex + LLPS, Dex + HLPS, LLPS and HLPS groups. In EAU, Inflammation of eyes in IRBP group was scored significantly higher than the Saline group ($P < 0.05$) and the peak at Day 14. The inflammation scores were significant different when compared to that in the Saline or Dex + IRBP groups ($p < 0.05$). Inflammatory and degenerative changes were detected in the retina tissue in IRBP group. The EIU and EAU models were successfully induced and the peak of inflammation was at 48 hours (EIU) or Day 14 (EAU). Dex effectively inhibited the inflammation in both models.

IRB Status: None

Disclosures:

LICHUN ZHONG: Employee relationship with Toxikon Corporation

P119

PHOSPHO-CORTACTIN AND CAVEOLIN COLOCALIZE TO PILS THAT ARE SITES OF ECM DEGRADATION

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Cortactin (Cort) is a distinguishing component of podosome- or invadopodia-like structures (PILS) which are focal sites of extracellular matrix (ECM) degradation. Involvement of PILS in regulating ECM turnover and aqueous outflow facility remains unclear. Studies were conducted to clarify component involvement in ECM regulation at PILS. We have previously shown that both MMP2 and MMP14 (Aga et al 2008) colocalize with cortactin at PILS. Here, DQ gelatin was perfused into human and porcine anterior segments and distinct areas of gelatin degradation were observed including in the juxtacanalicular (JCT) region. Some gelatin degradation was coincident with the MMP14 labeling. MMP14 staining was also associated with sites of degradation and both actin and vimentin, an intermediate filament protein were similarly localized. MMP2 and MMP14 immunostaining were higher in frontal sections of human TM from anterior segments perfused at 2x compared to at 1x pressure. Cav immunostaining was higher in eyes flown at 2x than at 1x pressure. Cav1 was distributed throughout the TM, whereas the preponderance of Cav2 was seen at the inner TM and JCT. In TM tissue, punctuate cortactin immunostaining was observed throughout the TM. Stretching TM cells for 5 minutes left pCort-466 immunostaining levels either unchanged or reduced, while pCort-421 and pCort-486 levels were increased. Labeling of pCort-421 was apparent at cell's periphery, whereas pCort 466 and 486 were present as punctuate areas in cell extensions and the cell body. A robust colocalization of caveolin was observed with internalized MMP2 tracked by labeling with specific MMP2 antibodies. ECM degradation in TM tissue and cells appears to be mediated by MMP2 and MMP14 in structures containing Cav, Cort and actin.

IRB Status: None

Disclosures:

MINI AGA: No financial relationships to disclose

P120

ON THE SEARCH FOR ADULT STEM CELLS IN THE TRABECULAR MESHWORK OUTFLOW PATHWAY OF THE PRIMATE EYE

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Cell number in the trabecular meshwork (TM) outflow pathways declines with age and glaucoma. It is unclear, if adult TM stem cells exist and whether they can compensate for TM cell loss. Adult stem cells reside in niches, divide rarely, and retain proliferation markers such as 5-bromo-2'-deoxyuridine (BrdU) for prolonged periods of time. Here, we searched for long-term BrdU-retention in the primate TM outflow pathways. Four cynomolgus monkeys were treated with BrdU (30 mg/kg body weight) for 4 weeks. Two animals were sacrificed immediately thereafter (group1), two animals were sacrificed four weeks after BrdU treatment (group2, long-term BrdU retention). The number of BrdU-positive cells was quantified in the different parts of the TM, the scleral spur, the operculum region, along the corneal endothelium in region of Schwalbe's line (SL), and in Schlemm's canal (SC) endothelium. The number of BrdU-positive cells was evenly distributed throughout the different regions of the TM. In contrast, the number of BrdU-positive cells in SC endothelial cells was significantly higher than in all other areas of the TM. SC BrdU-positive cells had characteristics of vascular endothelial cells and were immunoreactive for the endothelial marker CD31. In group2, TM and SC BrdU intensity was weaker, and the number of positive cells was smaller. In contrast, BrdU-staining in cells covering Descemet's membrane in the region of SL was as frequent and intense as in group 1 indicating long-term BrdU retention. Double labeling experiments with OCT 3/4 strongly supported the concept that SL cells with long-term BrdU retention are stem cells. Our findings provide evidence for the presence of cells with long-term BrdU retention in the primate outflow pathway. The cells are localized in SL which likely constitutes the stem cell niche. The cells might be able to compensate for the loss of TM and/or corneal endothelial cells.

IRB Status: None

Disclosures:

BARBARA BRAUNGER: No financial relationships to disclose

P121

INVESTIGATION OF ENUCLEATED MOUSE EYES IN ORGAN CULTURE

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Acute perfusion of ex vivo mouse eyes is a useful model for glaucoma research due to anatomical, physiological and pharmacological similarities to the human conventional outflow pathway, but only provides short-term assessments (~3hrs) of conventional outflow facility (C). Long-term assessments of outflow facility require organ culture perfusion that, while common for primate, porcine and bovine eyes, has not been developed for mice. Our goal was to investigate whether whole enucleated mouse eyes can be maintained in organ culture by simply submerging the eye in a glucose-containing saline bath, and we aim to characterize the effect of organ culture on outflow facility. Baseline C was measured immediately after enucleation in paired eyes from C57BL/6 mice (8-10 weeks), where C was defined as the slope of the flow rate-pressure data at 4, 8

and 15 mmHg. One eye of each pair was then "organ-cultured" via perfusion from a reservoir at 8 mmHg (without measuring C) for 18-20 hours, followed by a second facility measurement. The perfusion fluid was Dulbecco's phosphate buffered saline (PBS) + 5.5mM glucose + antibiotics (PSG), and the eyes were maintained at 35°C in a bath of PBS + PSG (for acute perfusion) or DBG + PSG (for organ culture perfusion). Baseline C for organ-cultured eyes was 32.5% larger than baseline C for acute-perfused eyes (0.0277 ± 0.0033 vs. 0.0209 ± 0.0042 $\mu\text{L}/\text{min}/\text{mmHg}$; mean \pm SD, $p=0.002$, $N=5$), suggesting a potential role for glucose in the bathing solution. After ~18hrs of culture, there was no significant change in C from baseline values (0.0277 ± 0.0033 vs. 0.0249 ± 0.0110 $\mu\text{L}/\text{min}/\text{mmHg}$; mean \pm SD, $p=0.606$, $N=4$). Organ-cultured eyes maintained C within normal limits despite 18+ hours of perfusion, suggesting that organ culture may be viable for whole mouse eyes. Future studies will examine the histologic changes and pharmacological response of organ cultured mouse eyes.

IRB Status: Verified

Disclosures:

JASON CHANG: No financial relationships to disclose

P122

PRESERVATION OF RETINAL GANGLION CELL FUNCTION BY TAUROURSODEOXYCHOLIC ACID

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Bear bile has been used traditionally in Chinese medicine for the improvement of visual acuity, but it has not been until recently that several investigations have demonstrated the anti-apoptotic properties of the bile constituent tauroursodeoxycholic acid (TUDCA) in animal models of photoreceptor degeneration. However, few studies have focused on visual disorders affecting other retinal cell types and, therefore, the aim of this work is to test whether systemic administration of TUDCA protects retinal ganglion cells from apoptosis triggered by excitotoxic insult. TUDCA efficacy was evaluated in an *in vivo* model of acute ganglion cell death, induced by intravitreal injection of N-methyl-D-aspartate (NMDA). A daily intraperitoneal dose of TUDCA (500 mg/kg) or vehicle (saline) was administered to adult Sprague Dawley rats for 1 week. At the fourth day, the animals received an intravitreal injection of NMDA (3 ml, 20 mM). Retinal ganglion cell functionality was addressed by electroretinogram recording of the positive and negative components of the scotopic threshold response (pSTR and nSTR), before and after NMDA-induced damage, just before sacrificed. Retinal tissue was harvested and processed for immunohistochemistry and confocal microscopy imaging. Staining of whole mount retinas with the specific ganglion cell marker Brn3a revealed a significant delay in NMDA induced apoptosis upon systemic TUDCA administration.

These data correlated with a more moderate decrease of both pSTR and nSTR responses in TUDCA-treated animals. TUDCA is currently under evaluation in several active clinical trials for various pathologies including cystic fibrosis, cholestasis, diabetes/obesity and amyotrophic lateral sclerosis. Our results sustain the efficacy of TUDCA in preventing retinal ganglion cell death, paving the way for clinical trials in glaucoma patients and other degenerative diseases coursing with retinal ganglion cell loss.

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IRB Status: International

Disclosures:

NICOLAS CUENCA: No financial relationships to disclose

P123

CRYSTAL STRUCTURE OF THE OLFACTOMEDIN DOMAIN OF MYOCILIN

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The protein myocilin is strongly linked with inherited forms of primary open angle glaucoma. Mutant myocilin aggregates within the endoplasmic reticulum of human trabecular meshwork cells leading to cell death and the misregulation of intraocular eye pressure, which is a hallmark of glaucoma. Over 90% of these mutations occur in the independently folding olfactomedin (myocilin-OLF) domain. Presented is the 1.9 Å resolution crystal structure of myocilin-OLF, the first known of any olfactomedin domain. The structure provides important insights into the location of disease-associated mutations and their effect on protein stability, the amyloidogenic regions of myocilin-OLF and the currently unknown function of both olfactomedin domains and native myocilin. This structure will also provide a starting point for structure based drug design for the treatment of myocilin glaucoma.

IRB Status: None

Disclosures:

REBECCA DONEGAN: No financial relationships to disclose

P124

SPHINGOLIPIDS AND CERAMIDES OF MOUSE AQUEOUS HUMOR: COMPARATIVE PROFILES FROM NORMOTENSIVE AND HYPERTENSIVE DBA/2J MICE

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To identify the sphingolipid and ceramide species and their quantitative differences between normotensive and hyper-

tensive intraocular pressure states in DBA/2J mouse aqueous humor (AH). Normotensive and hypertensive AH was sampled from mice by paracentesis. Lipid extraction was performed using modifications of the Bligh and Dyer method. Protein concentration was estimated using the Bradford colorimetric assay. Sphingolipids and ceramides were identified and subjected to ratiometric quantification using appropriate class specific lipid standards on a TSQ Quantum Access Max triple quadrupole mass spectrometer. The comparative profiles of normotensive and hypertensive DBA/2J mouse AH showed several species of sphingomyelin, sphingoid base, sphingoid base-1-phosphate (S1P) and ceramides common between them. A number of unique lipids in each of the above lipid classes were also identified in normotensive AH that were absent in hypertensive AH and vice versa. A number of sphingolipid and ceramide species were found to be uniquely present in normotensive, but absent in hypertensive AH and vice versa. Further pursuit of these findings is likely to contribute towards expanding our understanding of the molecular changes associated with increased intraocular pressure (IOP) and glaucoma.

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Disclosures:

GENEA EDWARDS: No financial relationships to disclose

P125

INVESTIGATING THE INFLUENCE OF BLAST ON CELLULARITY IN THE RETINAL GANGLION CELL LAYER IN A MOUSE MODEL OF BLAST-INDUCED TRAUMATIC BRAIN INJURY USING A SEMI-AUTOMATED TECHNIQUE

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Mouse models of disease have become an increasingly used and valuable resource in studies of glaucoma and traumatic brain injury (TBI). The visual deficits associated with these conditions are dependent upon the fate of retinal ganglion cells (RGC), however methods to quantify RGC are limited. The purpose of these experiments is to develop a robust method to quantify cellularity and investigate the influence of blast on the retinal ganglion cell layer (GCL) after blast-induced TBI. C57BL/6J mice were exposed to an overpressure wave using a custom-built blast chamber. Mice placed in the chamber without blast were used as sham controls. At 4 months post-blast, retinas from both blast-injured (n=16) and control (n=12) eyes were mounted whole, stained, and imaged by light microscopy. Images were uniformly collected

across the entire retinal area with equal sampling from the central and peripheral regions of the retina. Images were analyzed using custom-written macros for quantitative assessment of cellularity in the GCL. A semi-automated technique using ImageJ was developed that robustly quantifies GCL density. In retinas from both blast-injured and control mice, greater cell densities were observed in the central compared to the peripheral retina. In the peripheral retina, blast-injured mice exhibit a significant decrease in cell density compared to controls ($p = 0.03$, Student's t -test). In the central retina, blast-injured mice exhibit a trend of reduced cell density compared to controls. These results demonstrate that this mouse model of blast-induced TBI involves a loss of GCL cellularity by 4 months post-exposure. Using this model and our semi-automated quantification technique, our ongoing studies will test mechanisms contributing to this RGC susceptibility, with a long term goal of contributing to the development of improved clinical testing and treatment of visual deficits to those suffering from TBI.

IRB Status: Approved

Disclosures:

ADAM HEDBERG-BUENZ: No financial relationships to disclose

P126

TRANSCRIPTOME ANALYSIS OF LASER-CAPTURED RETINAL GANGLION CELL LAYER REVEALS ROBUST EXPRESSION AND ENRICHMENT OF CRYSTALLIN TRANSCRIPTS

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Developing novel therapies to replace or regenerate retinal ganglion cells (RGCs) for treating optic neuropathies requires a thorough understanding RGC development and maturation. To identify novel genes and splice variants involved in RGC development and maturation, the retinal ganglion cell layer (GCL) from C57Bl/6 mice (postnatal day 2) was isolated by laser capture microdissection (LCM) for whole transcriptome sequencing. Cryosections (7 μ m) were collected on PEN-membrane slides prior LCM (Arcturus XT) and RNA isolation ($n=4$ pooled RNA samples). RNA was also isolated from whole retina without LCM ($n=2$). Libraries for paired-end sequencing (Illumina HiSeq2000) were generated from total RNA (100ng/sample) and analyzed using Galaxy (<http://usegalaxy.org>). Each library contained between 37.2-43.3 million, high quality, paired reads, with 75-82% mapped to the mouse reference genome, with average quality scores above 30/base across all samples (0-low, 40-high). Known RGC-specific transcripts, *Pou4f2* and *Isl1*, were 5.6- and 2.6-fold higher in GCL respectively, based on gene transcript fragments/kilobase of exon/ million fragments mapped (FPKM) Photoreceptor-associated transcripts, *Crx* and *Nrl*, were 14.0- and 16.7-fold higher in whole retina vs. GCL respectively. Among transcripts enriched in GCL, 15 of the 100 most highly expressed transcripts encoded crystallins and heatshock proteins (52.3-fold average enrichment, GCL vs. whole retina). Results show enrichment of known RGC-specific transcripts and depletion of non-RGC transcripts in the GCL samples. The high expression and

enrichment of crystallin transcripts in the GCL at P2 suggest a possible role in GCL development. Interestingly, crystallin genes are up-regulated in glaucoma and may function in neuroprotection. Whole transcriptome sequencing of LCM tissue offers unique opportunities for gene discovery for understanding retinal development.

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IRB Status: Approved

Disclosures:

STEVE HUYNH: No financial relationships to disclose

P127

EFFECT OF IMMUNIZATION WITH OCULAR ANTIGENS ON EXTRACELLULAR MATRIX AND GLIAL CELLS

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RUHR-UNIVERSITY BOCHUM, CELL MORPHOLOGY AND MOLECULAR NEUROBIOLOGY²

Glaucoma is characterized by death of retinal ganglion cells (RGCs) and their axons. But its causes are not fully understood yet. In an autoimmune glaucoma model RGC loss can be induced by immunizing with ocular antigens. To gain further knowledge about remodeling of extracellular matrix (ECM) proteins and glial reactivity in this model, immunoreactivity against several proteins was evaluated. Rats were immunized with optic nerve homogenate (ONA) or S100 protein and compared to controls (CO). At 14 days RGC numbers were comparable in all groups, at 28 days fewer RGCs were observed in ONA and S100 retinas ($p<0.05$). Diverse effects were noted regarding ECM. At 7 days phosphacan reactivity was higher in ONA retinas ($p=0.0007$), its expression was mainly restricted to Müller glia processes. Phosphacan expression in S100 retinas was not altered ($p=0.09$). At 14 days phosphacan staining in ONA retinas further increased ($p=0.0007$). At this point activated microglia were observed ($p=0.0002$). Later, an increased expression of macroglia was also noted ($p=0.003$). S100 retinas were still not affected by phosphacan or macroglia changes ($p>0.05$). Tenascin-C expression was observed in the nerve fiber and the plexiform layers of the retina. Tenascin reactivity increased in both immunized groups at 7 days ($p<0.05$), while it went back to control levels later. Remodeling of the ECM components tenascin and phosphacan occurred shortly after immunization. Up-regulation of phosphacan in macroglia was continuously and exclusively noted in ONA, due to a reactive gliosis. The early up-regulation of tenascin was accompanied by retinal degeneration and microglia activation.

IRB Status: None

Disclosures:

STEPHANIE JOACHIM: No financial relationships to disclose

GAP JUNCTIONS AID DIFFERENTIATION OF INDUCED PLURIPOTENT STEM CELLS INTO TRABECULAR MESHWORK-LIKE CELLS

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Trabecular meshwork (TM) is responsible for the maintenance of intraocular pressure and has phagocytic properties for cleaning debris. Gap junctions play a critical role in cell differentiation and proliferation, and recently gap junctions were found to be expressed in various induced pluripotent stem cell (iPSCs) lines. Cellular loss in the TM of primary open angle glaucoma patients contributes to a loss of IOP homeostasis, which suggests that cell replacement to restore function might be an innovative glaucoma therapy. Previously (ARVO, 2014), we reported differentiation of iPSCs to a TM-like cell type, and their subsequent transplantation with restoration of function in a TM cell depletion model. In our earlier differentiation study, we used a specific combination of media conditions to differentiate iPSCs to TM-like cells, but the mechanisms involved are not totally clear. Here, we investigated the role of gap junctions in iPSC differentiated to a TM-like cell. These cells expressed the same markers as endogenous TM cells, including AQP1, Chi3L1 and MGP, and showed an absence of stem cell transcription factors OCT3/4, SOX2 and Nanog. Undifferentiated iPSCs expressed a gap junction protein, Cx43. The differentiated TM-like iPSCs and cultured human TM cells also expressed a high level of Cx43, but additionally, were capable of phagocytosis. Using the same differentiation conditions in the presence of the gap junction blocker Carbenoxolone (CBX), the differentiation of iPSCs to a TM-like cell was blocked, as shown by a decrease in mature TM marker expression, and the presence of stem cell transcription factors. Furthermore, with the addition of CBX, the cells expressed a lower level of Cx43 and did not phagocytose. This use of the gap junction blocker CBX suggests that gap junctional communication, mediated by Cx43, may play an important role in the differentiation of iPSCs to TM like cells.

IRB Status: Verified

Disclosures:

MARY KELLEY: No financial relationships to disclose

EFFECTIVE AND PROSPECTIVENESS OF SELECTIVE LASER TRABECULOPLASTY IN TREATMENT OF PRIMARY OPEN ANGLE GLAUCOMA PATIENTS

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MEDICAL CENTER MTZ

According to WHO glaucoma is the significant problem in present-day ophthalmology that appears to be one of the main reasons of incurable vision loss. Over 15% of blind people in the world have lost eyesight in glaucoma. The purpose of present

work was to study the data of investigations of selective laser trabeculoplasty (SLT) application in treatment of primary open angle glaucoma patients. 92 patients – 59 males and 33 females (121 eyes) – at the age from 41 to 85 years with primary open angle glaucoma (POAG) were under medical observation. Selective laser trabeculoplasty was performed on Nd: YAG Laser Selecta Duos (Lumenis). First group of patients (50 eyes) underwent 100 laser applications (in all segments of corneal trabecula – 360°). In the second group (71 eyes) 50 laser applications (0.8 – 1.2 mJ) in inferior segment were performed (180°). Statistically significant ($p < 0.05$) decrease of intraocular pressure (IOP) after SLT was noted in both groups. To the first month this reduction was 3.4 mm Hg in the first group and 1.5 in the second. To the third month – 3.9 and 2.8 respectively. To the sixth month – 3.8 and 2.5 respectively. Long effect of SLT was noted in 82% cases in the first group (360°) and 74.6% - in the second (180°). Selective laser trabeculoplasty is safe and effective method of reducing IOP in patients with POAG I-II stages.

IRB Status: None

Disclosures:

GLEB KRISHTOPENKO: No financial relationships to disclose

PRIMARY OPEN ANGLE GLAUCOMA AND PHACOEMULSIFICATION

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MEDICAL CENTER MTZ, OPHTHALMOLOGY

To evaluate intraocular pressure (IOP) in patients with primary open angle glaucoma (POAG) 1-2 stages before and 1 month after phacoemulsification (PE) according to the anatomical parameters of the eye. The study included 55 eyes with POAG stage 1-2 and cataract, which was performed PE and 36 is not operated eyes (control group). In all eyes we measure IOP before and 1 month after PE by Maklakov tonometry.

Among all the operated eyes mean IOP decreased from -19.27 ± 5.24 mm. Hg to 16.76 ± 5.64 ($p < 0.05$); size of the lens -4.56 ± 0.61 mm, mean size of eye - 23, 17 ± 1.42 mm. In 65% cases IOP decreased and in 35% - increased.

On eyes with size less than 22 mm (7 eyes) IOP was 17.28 ± 4.57 mm Hg, after 1 month- 19.42 ± 9.07 mm ($P > 0,05$).

On eyes with size 22-23 mm. (20 eyes) IOP was 17.3 ± 5.71 mm, after 1 month 16.9 ± 5.87 mm ($p > 0,05$).

On eyes with size more 23 mm IOP decreased from 21.17 ± 4.44 mm to 16.03 ± 4.33 mm. Average reduction 5.17 mm Hg ($P < 0,05$).

Size of the lens - 4.46 ± 0.61 mm size of eye - 23, 17 ± 1.42 mm. In 65% cases IOP. On eyes with size 22-23 mm (20 eyes).

On eyes with size more 23 mm IOP decreased from 21.17 ± 4.44 mm. In control group IOP not changed. Performing PE in patients with POAG is accompanied by changes

IRB Status: None

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P131

DIFFERENCE IN RELATIONSHIP BETWEEN CHOROIDAL THICKNESS MEASURED BY CIRRUS OCT AND HEMODYNAMIC PARAMETERS AMONG NORMAL SUBJECTS AND NORMAL TENSION GLAUCOMA PATIENTS

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Aim of this study is evaluate difference in relationship between Choroidal thickness measured by Cirrus OCT using Evaluate Enhanced depth Imaging mode and hemodynamic parameters among normal subjects and normal tension glaucoma patients (NTG). In this cross-sectional retrospective study, medical records of 85 eyes (47 normal and 37 with NTG) from 85 patients were reviewed. All subjects did not use any medications to control intraocular pressure and blood-pressure within 1 month before tests. Choroidal thickness were measured at 6 points around optic disc and at 5 points in macular area. Blood-pressure and IOP and ocular pulse amplitude measured by Goldman applanation tonometry and dynamic contour tonometry were measured same day that OCT was taken. Hemodynamic parameters and Choroidal thicknesses were compared between normal and NTG patients group and statistical relationship among parameters. In our study, IOP, axial length, spherical equivalent, central corneal thickness and systolic and diastolic blood pressure were not different between normal and NTG group. Average peripapillary and macular choroidal thickness were $200.5 \pm 79.9 \mu\text{m}$ and $275.2 \pm 87.9 \mu\text{m}$ in normal group and $188.7 \pm 84.6 \mu\text{m}$ and $248.3 \pm 78.4 \mu\text{m}$ in NTG respectively. Choroidal thickness at 6 Peripapillary and 5 macular points also showed no significant differences. Average peripapillary choroidal thickness negatively correlated with systolic ($r = -0.422$, $p = 0.009$) and diastolic pressure ($r = -0.419$, $p = 0.010$) but, there were no significant relationship in normal group. In conclusion, there were no differences choroidal thickness between normal and untreated NTG patients. But relationship between choroidal thickness and hemodynamic parameters were not same among normal and NTG. Unlike normal subjects choroidal thickness of NTG patients was influenced by blood pressure. These findings can be another clue of compromised blood flow to explain pathogenesis of NTG.

IRB Status: Approved

Disclosures:

MARVIN LEE: No financial relationships to disclose

P132

FUNCTIONAL CELLULAR CONSEQUENCES OF AGEING IN PORCINE ANGULAR AQUEOUS PLEXI

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The purpose of this study was to investigate the effect of ageing on barrier function of porcine angular aqueous plexus (AAP) cells, the porcine equivalent of human Schlemm's canal cells, exposed to pressure gradients. AAP cells were differentially isolated from porcine outflow tissues using puromycin selection. Confluent cultures of AAP cells were grown for 2 weeks in physiological (5% O₂) or hyperoxic conditions (40% O₂) to model ageing/senescence. Control and aged AAP cells were subjected to control and elevated hydrostatic pressure gradients (0.4 and 10 mmHg) for 72 hours, perfused in basal to apical direction. Cell transendothelial electrical resistance (TEER) and hydraulic conductivity (HC) were measured. Data showed that ageing alone resulted in a significant 30% increase in TEER compared with control ($p < 0.05$, $n = 6$). When perfused in the basal-to-apical direction at 4 mmHg, HC of AAP cells was significantly reduced in aged cells (from 1.97 ± 0.12 to $1.54 \pm 0.13 \text{ uL/mmHg/min/cm}^2$, $p < 0.05$, $n = 6$). Aged cells also expressed a significantly greater abundance of F-actin, phospho-myosin light chain (MLC), occludin, claudin-5, beta-catenin and VE-cadherin compared to the control by both immunofluorescence and western blot analyses. Pressure elevation resulted in a significant increase of HC in control cells (from 1.37 ± 0.12 to $1.64 \pm 0.18 \text{ uL/mmHg/min/cm}^2$, $p < 0.05$), but not in senescent cells. TEER changes were consistent with the HC results that it was significantly lower in control cells (28 ± 2.4 vs. $22 \pm 3.2 \text{ ohms} \cdot \text{cm}^2$, $p < 0.05$) but not in aged cells. Western blot analysis showed that the expression level of cell junction proteins was significantly reduced after exposure to pressure elevation in control cells but not in aged cells. We conclude that ageing not only increased resistance of AAP monolayers but also rendered the cells less able to respond to pressure elevation, which may have pathological consequences that lead to ocular hypertension.

IRB Status: International

Disclosures:

YUAN LEI: No financial relationships to disclose

P133

TIGHT JUNCTION PROTEIN CLAUDIN-1 IS A MARKER FOR SCHLEMM'S CANAL CELLS

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Primary open-angle glaucoma (POAG) is one of the leading causes of blindness. Intraocular pressure (IOP) elevation, common in POAG, results from increased resistance to aqueous humor (AH) outflow and is one of the key risk factors for optic nerve damage. Although great progress has been made in studying the pathogenesis of POAG, the mechanisms that cause this disease are not yet fully understood. Trabec-

ular meshwork (TM) and Schlemm's Canal (SC) cells play a critical role in the regulation of aqueous flow resistance. Gap and tight junctions are important structures for cell to cell communication or for forming barrier functions between cells. Previously, others demonstrated gap and tight junctions in the inner wall of SC by freeze fracture and quick freeze deep-etch electron microscopy, and some studies have been conducted on the junctional proteins. In other tissues, claudin-1 and connexin43 (Cx43) are major tight and gap junction proteins, respectively, and both are associated with the protein ZO-1. Here, we investigated claudin-1, Cx43, and ZO-1 expression in cells from TM and SC of human cadaver and porcine eyes. SC cells were isolated with magnetic beads using antibodies to PECAM-1, an established SC marker. Cells from sections of perfused human eyes and porcine primary cultured cells were immunolabeled for claudin-1, Cx43, and ZO-1. Cx43 and ZO-1 were detected in TM and SC by immunohistochemistry and confocal microscopy, but claudin-1 was expressed only in SC. Claudin-1 and PECAM-1 both localized to SC. In summary, the tight junction protein claudin-1 is restricted to SC, and not present in TM. Thus, this protein is an additional biomarker for SC. Additionally, SC, with its continuous tight junctions and tight junction protein claudin-1, may have a unique functional responsibility in the JCT/SC inner wall complex, potentially contributing to the resistance to aqueous humor outflow.

IRB Status: Verified

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XINBO LI: No financial relationships to disclose

P134

A CASE OF BENIGN NMO SPECTRUM DISORDER DURING PREGNANCY

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Neuromyelitis optica spectrum disorder (NMO-SD) is an autoimmune inflammatory disorder known to be related with anti-aquaporin-4 (AQP-4) antibody. Over 90% of the NMO-SD patients follow a severe clinical course and the pregnancy is known to be a disease-worsening factor. Here we report a case of NMO-SD that recovered naturally during pregnancy. A 29-years-old woman developed optic neuritis (ON) of the right eye on 14 weeks of her pregnancy. Her best correlated visual acuity (BCVA) was 0.02, the critical flicker frequency (CFF) was not measurable, and the mean deviation (MD) of Humphrey 30-2 was -42 dB. MRI (T2 STIR) showed a high signal in the right optic nerve. Also the patient was seropositive with anti-AQP4 antibody. Patient was diagnosed with NMO-SD by presence of the optic neuritis and seropositive anti-AQP-4 antibody. Corticosteroid pulse therapy was considered, but the patient denied any treatment since it was in first trimester of her pregnancy. Two weeks later, her BCVA had improved to 1.2 (OD). Nine weeks later, the MD of Humphrey 30-2 improved to -1.2 dB, and the CFF also increased to 39.8Hz. There has been no sign of recurrence for 6 months. Natural recovered cases of NMO-SD during pregnancy are very rare.

Although the factors of good outcome have not been identified, this case suggests that the early stage of the pregnancy may have different immunological condition that could affect the clinical course of NMO-SD. Further studies are needed to identify the relationship between pregnancy and NMO-SD.

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Disclosures:

RYUTARO AKIBA: No financial relationships to disclose

P135

THE V-DOMAIN IG SUPPRESSOR OF CELL ACTIVATION (VISTA) PLAYS AN ESSENTIAL ROLE IN THE ACCEPTANCE OF CORNEAL ALLOGRAFTS

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NIPPON MEDICAL SCHOOL¹; TOKYO MEDICAL AND DENTAL UNIVERSITY²

V-domain Ig suppressor of T cell activation (VISTA) is a novel and structurally distinct Ig superfamily inhibitory ligand. We have previously demonstrated that survival of allografts treated with anti-VISTA mAb was less than that of the control, and that VISTA plays important role in induction of alloantigen-specific ACAID. To further investigate the mechanism of VISTA-mediated corneal allograft survival, we examined destruction of corneal endothelial cells (CECs) by allo-reactive T cells in vitro. The corneas from C57BL/6 (B6) eyes pre-treated with anti-VISTA mAb or control rat IgG were incubated with CD4+ T cells. Dead CECs stained with propidium iodide were counted and compared. No significant differences were observed between the number of dead CECs treated anti-VISTA monoclonal antibodies (mAb) and that treated control IgG after incubation with allo-reactive T cells. It is indicated that VISTA does not have protective effect in the cornea from the allo-specific killing by CD4 T cells. As the next, we examined infiltrating T cells in the graft-bearing eyes from the recipients treated with anti-VISTA or control IgG. Corneas of C57BL/6 were transplanted into normal eyes of BALB/c mice. Recipients were administrated with anti-VISTA mAb or control rat IgG. Immunofluorescent staining of the graft-bearing eyes at 3 to 5 weeks after grafting revealed that the numbers of infiltrating CD4 and CD8+ T cells at graft center and host-graft junction were significantly higher in anti-VISTA treated recipients compared to control. Taken together of our present and previous data, it is suggested that VISTA plays an role in the acceptance of corneal allografts by inducing allo-specific ACAID which suppress T cell infiltration into the cornea, although VISTA doesn't have a local protective effect in the interaction between CECs and CD4+ T cells.

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REGULATION OF THE MICROGLIAL PHAGOCYTOSIS-SENSOR TREM2 (CHR6P21) BY AN NF-KB-SENSITIVE MIRNA-34A (CHR 1P36) IN AGE-RELATED MACULAR DEGENERATION (AMD)

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Progressive amyloid deposition and inflammatory degeneration in the human retina is associated with aberrant innate-immune signaling in age-related macular degeneration (AMD). Genetic deficits and loss-of-function for the triggering receptor expressed in myeloid/microglial cells 2 (TREM2; encoded at chr6p21.1), a transmembrane spanning, phagocytosis-sensing stimulatory receptor of the immunoglobulin/lectin-like gene superfamily, have been associated with deficiencies in phagocytosis, innate-immune system signaling and amyloidogenesis. In this report we provide evidence that TREM2 expression is down-regulated in the retina in dry AMD compared to age-matched controls. An NF- κ B-sensitive miRNA-34a (encoded at chr1p36.22), up-regulated in AMD, was found to target the 299 nucleotide human TREM2 mRNA 3'-UTR, and down-regulated the expression of a TREM2-3'-UTR reporter vector transfected into C8B4 microglial cells. Anti-NF- κ B agents, including the resveratrol analog CAY10512, the anti-inflammatory bee resin-derived caffeic acid phenethyl ester (CAPE) or the natural phenolic antioxidant curcumin (diferuloylmethane), and stabilized anti-miRNA-34a (AM34a) strategies were found to quench this pathogenic response. The results indicate that an epigenetic mechanism involving an NF- κ B-mediated, miRNA-34a-regulated down-regulation of TREM2 expression may shape innate-immune and phagocytic responses that contribute to inflammatory degeneration of the retina and other progressive age-related disorders with an amyloidogenic component. These data also suggest that highly specific mechanisms involving extremely selective, inducible miRNAs contribute to immune deficits and pro-inflammatory signaling characteristic of progressive, age-related disorders, and support the use of novel transcription factor- and nucleic acid-based therapeutic strategies in the clinical management of AMD.

IRB Status: Approved

Disclosures:

WALTER LUKIW: No financial relationships to disclose

THE POSSIBLE ROLE OF BRADYKININ IN OCULAR INFLAMMATION: UVEITIC MACULAR OEDEMA AS AN EXAMPLE

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Macular oedema is a frequent complication of uveitis regardless of its aetiology. It is primarily characterised by the accumulation of fluid in the retinal layers around the fovea with an annual incidence between 17-52 cases per 100,000 populations. Inflammatory mediators, such as kinins, released during uveitis have been suggested to play a role in the formation and progression of macular oedema, however the mechanism is not understood. Kinins, such as bradykinin, are bioactive peptides released at the sites of tissue damage, either in response to stimuli, such as trauma or infection, or during inflammation. Kinins mediate most of their effects through the stimulation of two different G-protein-coupled receptors, classified as B1 and B2 receptor. This study investigates the role of bradykinin and its receptors in human ocular inflammation. Aqueous humour was collected from patients with uveitis (n=9) and fourier transform mass spectrometry (FT/MS) was performed to screen for inflammatory mediators (bradykinin and kininogen). Immunohistochemistry was used to investigate expression of bradykinin B1 and B2 receptors from human ocular tissue, iris and retinal pigment epithelial cells. ELISA was used to measure bradykinin from cultured supernatants of LPS treated pigment epithelial cells. Bradykinin and its precursor, kininogen, were detected in the aqueous humour of patients with uveitis by mass spectrometry. Of the nine patients two had raised bradykinin levels and four had increase kininogen levels. Expression of bradykinin B2 receptor was seen in human iris and retina as well as in iris and retinal pigment epithelial cells. However, bradykinin was not detected from cultured supernatants. This could be due to a short half-life of bradykinin. Expression of bradykinin B2 receptors in human iris, retina and ocular pigment epithelial cells suggests a role in ocular inflammation and its activation may be involved in the pathogenesis of macular oedema.

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Disclosures:

KELLY MAI: No financial relationships to disclose

HERPES SIMPLEX VIRUS-1 STRAIN KOS INDUCES CLINICAL AND IMMUNOLOGICAL CHANGES IN ACUTE HERPETIC KERATITIS

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Virulence of herpes simplex virus type-1 (HSV-1) strain KOS to induce acute keratitis remains controversial. We aimed to investigate acute clinical and immunological corneal responses to KOS inoculation. Corneas of 6-10 week-old female C57BL/6 mice were inoculated with 2×10^4 (low titer, LT) and 2×10^6 PFU (high titer, HT) of HSV-1 KOS. Mice were scored every other day until 7 days post-infection (dpi) for blepharitis, corneal opacity, neovascularization and epitheliopathy. Virus titers were assessed by standard plaque assay. Whole-mounted corneas were stained for CD45 and beta tubulin at 1 and 7 dpi. On 3 dpi, flowcytometry was performed on corneal single cell suspensions for CD45 (pan-leukocyte), CD11c (dendritic cell), GR-1 (neutrophils), F4/80 (macrophages), B220, PDCA-1 (plasmacytoid dendritic cell) and NK-1.1 (natural killer cell) markers. On 7dpi with HT and LT infection, blepharitis score was 3.4 and 2.7, corneal opacity score was 3 and 1.5, and corneal neovascularization was 7.6 and 1.5 clock hours, respectively ($p=0.001$). Corneal epitheliopathy was the largest on 3 dpi (26.8% and 27.2% for HT and LT, respectively). Central corneal nerve decreased significantly at 1dpi (55.5%) and 7 dpi (complete absence). Corneal virus titers were the highest at 1 dpi (1.4×10^5 PFU/ml) and nothing detected on 7dpi. Virus titers in trigeminal ganglia were highest on 3dpi (1.7×10^6 PFU/ml) and declined slowly through 7 dpi (3.2×10^3 PFU/ml). Compared to normal cornea (periphery 262 cells/mm², center 178), CD45+ cells increased on 1 dpi (1123 cells/mm² periphery and 312 center) and 7 dpi (1470 cells/mm² periphery and 1237 center), ($P<0.05$). Flowcytometry revealed significant increase in all the stained immune cells. In summary, we demonstrated that KOS strain results in acute HSV-1 keratitis in a dose-dependent fashion, and induces a significant corneal inflammatory response and nerve damage, thus may be used as a model for future studies.

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Disclosures:

HAMIDREZA MOEIN: No financial relationships to disclose

P139

LONG-TERM OBSERVATION OF MURINE MODELS OF ANTERIOR SCLERITIS

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NIPPON MEDICAL SCHOOL

We have previously established a model of anterior scleritis by modifying a collagen-induced autoimmune arthritis model. In the present study, the long-term observation of the clinical and the immunological findings were performed. Male DBA/1J mice (8-week-old) received primary immunization in the back of the neck with 200 μ g of bovine type II collagen (CII) emulsified using equal volume of complete Freund's adjuvant (CFA, containing 100 μ g H37Ra *Mycobacterium tuberculosis*). After 3 weeks, CFA-emulsified CII was injected intradermally around the eye for secondary immunization, then the arthritis and eyes were examined. Eyeballs were excised at 3, 5, 8, 12 and 24 weeks after secondary immunization and analyzed histologically and immunohistologically. Clinical findings comprised severe arthritis and dilation of scleral blood vessels from 3 weeks

after secondary immunization. Histological findings revealed anterior scleral thickening, with significantly large number of infiltrating cells as compared to untreated mice. Infiltration of CD4+, CD11b+ cells were present in the Tenon's layer, while deposition of plasma cells (CD138), complement (C3), immunoglobulin (Ig)G and IgM were seen in the anterior sclera in contact with the ciliary body and blood and lymphatic growth (CD31 and LYVE-1) expression was increased in the corneal limbus compared to untreated mice, throughout all observation periods. T cells, macrophages, plasma cells, complement, immunoglobulins, the outgrowth of blood and lymphatic vessels were persistently found in the sclera of the collagen-induced anterior scleritis model. It is suggested that the involvement of immunocomplex deposition, and blood and lymphatic growth in the sclera is one of immunopathology of this scleritis model.

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P140

ADAMTS9 AS A CANDIDATE GENE IN PATHOGENESIS OF ANTERIOR SEGMENT DYSGENESIS

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Anterior Segment Dysgenesis (ASD) encompasses genetically heterogeneous disorders of ocular anterior segment (AS) morphogenesis that cause severe visual impairment in infants and children. ADAMTS9 is a secreted metalloprotease having multiple thrombospondin type I repeats (TSRs), which are post-translationally modified by O-fucosylation. Although *Adamts9* null (*del/del*) embryos die before initiation of eye development, we identified a highly penetrant ASD in *Adamts9del/+* mice. Peters anomaly in these eyes linked ADAMTS9 to human Peters-Plus syndrome (PPS) resulting from defects in the enzyme B3GALT1, which is involved in O-fucosylation of TSRs. OCT and histology of newborn and juvenile *Adamts9del/+* eyes consistently identified Peters anomaly, leakage of γ -crystallin positive lens material posteriorly, perturbed lens epithelium polarity, aberrant nests of lens epithelium and duplication of the anterior lens capsule. Staining intensity of collagen IV and laminin, the major lens capsule components, was reduced. Preliminary analysis of mice with vascular endothelium-specific conditional deletion showed similar lens anomalies, but as yet no other AS defects. *Adamts9* mRNA expression during eye development, ascertained by intragenic LacZ reporter and ISH, was observed in hyaloid vasculature, and the anterior pole of the optic cup. The hyaloid vasculature showed enhanced fibronectin and fibrillin-2 staining in *Adamts9del/+* eyes, suggesting these molecules as potential ADAMTS9 substrates. ADAMTS9 sequencing in ASD patients

has not hitherto identified causative mutations. The work to date identifies novel alterations in ocular extracellular matrix resulting from deleting ADAMTS9, and the novel concept that ADAMTS9 derived from hyaloid vasculature may contribute to lens development. Collectively, these studies have a strong likelihood of defining new pathways of AS development and new genetic insights on PPS-like conditions.

IRB Status: Approved

Disclosures:

JOHANNE DUBAIL: No financial relationships to disclose

P141

NANOSCALE TOPOGRAPHY AFFECTS TGF β -INDUCED EMT OF LENS EPITHELIAL CELLS

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Epithelial-mesenchymal transition (EMT) is a pathological process leading to the formation of posterior capsular opacification (PCO). The cytokine TGF β is known to induce EMT in lens epithelial cells (LEC's), resulting in the expression of the marker α -smooth muscle actin (α SMA). In other systems, surface topographic cues have been shown to affect the transformation of myofibroblasts from corneal stromal cells in response to TGF β . This study sought to determine if nanoscale surfaces alter the response of lens epithelial cells to TGF β treatment. Human lens epithelial FHL124 cells were seeded onto patterned polymeric (NOA81) chips at a density of 10,000 cells/cm². Chips with a groove pitch of 400nm or 1400nm were adhered to a 60mm culture plate and phase microscopy was used to observe the morphology of the plated cells during growth phase. Once confluency was reached, the cells were treated with 4ng/ml recombinant human TGF β 2. Cell lysates were harvested after 48 hours and protein expression was compared between treatments using Western blotting. Cells grown on 1400nm pitch surfaces show clear parallel alignment to the grooves of the topographic features, prior to reaching confluency. There was no difference in α SMA expression between untreated FHL cells grown on 400 and 1400nm pitch chips, compared to control surfaces. However, following TGF β treatment for 48 hours, LEC's grown on the pitch surfaces expressed less α SMA than TGF β -treated cells grown on flat controls, with 1400nm exhibiting the greatest reduction in the EMT marker. This study demonstrates the response of LEC's to TGF β stimulation is directly affected by growth surface topography. Specifically, cells grown on surfaces with a 1400nm pitch showed a decrease in expression of α SMA compared to controls when exposed to TGF β . Further investigation into how nanoscale topography, as well as other biophysical cues, affect LEC phenotype may lead to novel approaches to prevent PCO.

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SCOTT BOWMAN: No financial relationships to disclose

P142

A ROLE FOR HEDGEHOG SIGNALING DURING LENS AND CORNEAL DEVELOPMENT

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Previous studies suggest Hedgehog (Hh) signaling plays roles in ocular development. In this study, we investigated the expression and requirement for Hh signaling in ocular development using conditional gene knockouts. Hh pathway expression was investigated by RT-PCR, immunofluorescence and *in situ* hybridisation. The requirement of *Smoothened* (*Smo*) was investigated by conditional loss-of function in embryonic mouse eyes by crossing *Smofl/fl* mice with LeCre and MLR10 Cre mice. The phenotype of mutant mice was examined by immunofluorescence for various markers of cell cycle, lens and cornea differentiation. Distinct expression of *Smo*, *Ptch1*, *Gli2* and *Gli3* were detected in lens epithelium from E12.5. *Gli2*, an activator of downstream genes showed distinct association with M-phase chromosomes. By contrast the repressor, *Gli3*, shifted from cytosol to nucleus, suggesting suppression of Hh signals from E13.5. Consistent with this, deletion of *Smo* using MLR10 Cre (active from E12.5) showed no lens defects. However, deletion with LeCre (active from E10.5) resulted in lens and anterior segment defects from E14.5. Mutant lenses showed a deficient epithelium with defects of G2/M phase, decreased FoxE3 expression and increased apoptosis. No major changes in fibre cell markers were detected. Corneal endothelial but not epithelial differentiation appeared delayed in mutant corneas at E16.5. Mutant embryonic corneas were thicker due to aberrant migration of Nrp2+ (semaphorin receptor) cells from the extraocular mesenchyme, resulting in delayed endothelial differentiation. While no gross changes in *Sema3A* or *Sema3F* expression were detected in LeSmoX lenses, a similar thickened corneal phenotype was found in mice that lack *Sema3A*. The Hh pathway is required during a discrete period (E10.5-E12.5) in lens development to regulate lens epithelial cell proliferation, survival and FoxE3 expression. Defects detected in the cornea, secondary to defects in lens, may be related to abnormal signaling from the lens.

IRB Status: None

Disclosures:

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P143

SEQUENCE – STRUCTURE – FUNCTION OF LENS CRYSTALLINS

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IMPIC – CNRS/IUPMC, BIOINFORMATICS AND BIOPHYSICS

The major soluble proteins in the vertebrate eye lens, alpha-beta- and gamma-crystallins, are fundamental for maintaining the necessary transparency of the lens. The lens transparency,

enhanced by the elimination of organelles (like mitochondria and nuclei) in the postmitotic fiber cells, is due to high concentration of these lens proteins that present exceptional longevity. Gamma-crystallins are monomeric whereas beta-crystallins are hetero-oligomeric. Alpha-crystallins, belonging to the small heat shock protein superfamily, form large oligomers. Their function, known as an ATP-independent chaperone activity, is to protect the other proteins from various cellular stress and it consists to form soluble complexes with various target proteins, avoiding their aggregation, potentially nocive for the cell. The oligomer formation and the chaperone activity is related to a dynamical structure, ie the ability of these proteins to exchange their subunits with themselves or with other proteins. The structure-function relationship of different crystallins, native and mutant corresponding to human pathologies, were studied by biochemical and structural biology approaches, comparing the assembly formation and function depending upon various stress conditions. Our results have showed a direct correlation between the modification of oligomeric formation, the subunit exchange and the loss of function in vitro.

IRB Status: None

Disclosures:

STÉPHANIE FINET: No financial relationships to disclose

P144

CHARACTERIZATION OF THE CHILDHOOD LAMELLAR CATARACT IN TRANSGENIC MICE: IMPAIRMENT OF SECONDARY FIBER CELLS MORPHOGENESIS

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JULES STEIN EYE INSTITUTE, UCLA¹; MOLECULAR AND MEDICAL PHARMACOLOGY, UCLA²; JULES STEIN EYE INSTITUTE, MOLECULAR BIOLOGY INSTITUTE AND BRAIN RESEARCH INSTITUTE, UCLA³

Transmission of light to the retina is critical for the neural development of the infant. About half of all congenital cataracts are inherited. Among these, the lamellar cataract, a bilateral condition is the most prevalent form of childhood cataracts. Unlike age-related cataracts, surgeries in children entail a lifelong follow-up and impaired vision. There are no paradigms for the study of early childhood cataract. Mutations in the DNA binding domain (DBD) of the heat shock transcription factor, HSF4, have been shown to associate with lamellar cataract. Based on these observations and employing recombineering to manipulate HSF4 gene in bacterial artificial chromosomes (BACs), we have we have recreated the human lamellar cataract in transgenic mice. The cataract in the transgenic mice is bilateral and morphologically confined to the nucleus as in the human pathology. The cataract phenotype presents as specks and streaks or spots involving a few lamellae. Histologic and biochemical characterization of the developing cataract in the postnatal day 2 mouse suggest an impairment of the secondary fiber cell differentiation concurrent with the loss of vimentin and Fgf7 expression. Three different transgenic mice lines (produced from three different BACs of various sizes, 160 - 228 kb) present a common pathology in the persistence and increase of secondary fiber cell nuclei in the developing lens suggesting

a block in the terminal differentiation of the fiber cells. While these transgenic mice, for the first time allow a molecular insight into childhood cataractogenesis in a detail that has not been possible previously, they also reveal the conserved role of the heterogeneity of the expression of HSF4-controlled gene activity in individual fiber cells within the lens.

This work was supported by NEI/NIH grants to SPB.

IRB Status: Approved

Disclosures:

RAJENDRA GANGALUM: No financial relationships to disclose

P145

DNMT1 EXPRESSION IS REQUIRED FOR LENS EPITHELIAL CELL SURVIVAL

EVAN HOROWITZ, Thanh Hoang, Blake Rasor, Blake Chaffee, Michael Robinson

MIAMI UNIVERSITY, DEPARTMENT OF BIOLOGY

Epigenetic mechanisms involving DNA methylation have an integral role in the regulation of gene expression during the process of cellular differentiation. Patterning of DNA methylation are used as a benchmark for determining induced pluripotent stem cell progression towards embryonic stem cell equivalents and aberrant patterning has been associated with forms of cancer. The catalytic function of DNA methyltransferase 1 (DNMT1) is to maintain methylation marks through subsequent rounds of DNA replication by methylating corresponding cytosines on the newly synthesized strand. To determine the role of Dnmt1 in lens development, *Dnmt1* was deleted from the lens lineage by crossing mice carrying a Cre-dependent deletion allele of *Dnmt1* (*Dnmt1*^{1L}) to Le-Cre transgenic mice. By E12.5 *Dnmt1*-deficient lenses displayed a small lens with fewer lens epithelial cells. Furthermore, the bow region of these Dnmt1-deficient lenses shifted anteriorly relative to the control lenses at E12.5. Although DNMT1 protein was undetectable in the lens by E12.5, lens epithelial cells still entered S-phase, but these cells also experienced increased cell death. Primary lens fiber cell differentiation markers p57Kip2, γ - and β -crystallins exhibited normal expression patterns when compared to the control. Although, elongation of primary lens fiber cells was delayed. As development progressed the number of lens epithelial cells continued to decline with the entire epithelium disappearing by approximately E15.5. The loss of the lens epithelium at E15.5 was accompanied by the formation of vacuoles within the fiber cell mass with subsequent degeneration of the entire lens. Delayed eyelid closure was also associated with the loss of Dnmt1. In summary, the loss of Dnmt1 (mediated by Le-Cre) resulted in the loss of the proliferating population of lens cells followed by lens degeneration.

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IRB Status: Approved

Disclosures:

EVAN HOROWITZ: Grant Support relationship with Miami University

LECS FROM MMP-9 KO MICE SHOW RESISTANCE TO TGF β -INDUCED EMT

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MCMaster UNIVERSITY¹; MCMaster UNIVERSITY, PATHOLOGY AND MOLECULAR MEDICINE²

Transforming growth factor beta (TGF β)-induced epithelial-mesenchymal transition (EMT) is involved in the formation of anterior subcapsular cataracts as well as secondary cataract. Our previous work suggests that of the two matrix metalloproteinases upregulated in this process, MMP-2 and -9, it is MMP-9 that appears to be the more critical mediator of TGF β -induced EMT. Here we further investigate the unique role of MMP-9 in mediating EMT. Lens epithelial explants, with epithelia attached to their native lens capsule, were isolated from MMP-2 and -9 wild-type and KO mice and maintained in culture. Confluent LEC explants were stimulated with TGF β for 48 hours and immunostained for F-actin, E-cadherin, β -catenin and α SMA. E-cadherin levels were quantified by ELISA and Western blot. Isolated lens epithelial explants from wild-type and MMP-2 KO mice that were treated with TGF β exhibited features indicative of EMT, namely a distinct loss of E-cadherin expression at the cell junctions, cytosolic β -catenin, stress fibre formation and significant α SMA expression. In contrast, MMP-9 KO explants even in the absence of TGF β stimulation exhibited irregular E-cadherin staining at cell junctions, but maintained comparable levels of total E-cadherin protein as compared to wild-type controls. Importantly, upon TGF β stimulation, MMP-9 KO explants did not acquire a characteristic mesenchymal phenotype resembling untreated controls with marginal β -catenin, cortical F-actin and absence of α SMA expression. The current findings demonstrate that MMP-9 KO mouse lens explants are resistant to TGF β -induced EMT, including the absence of stress fibre formation, and β -catenin de-localization, as well as α SMA expression. The abnormal E-cadherin localization and patterning attributed to the lack of MMP-9 will need to be further explored and may be a key factor in the protection of these explants from TGF β -induced EMT.

IRB Status: None

Disclosures:

ANNA KOROL: No financial relationships to disclose

UV-TREATMENT OF THE RAT EYE INCREASES CRYSTALLIN EXPRESSION IN THE RETINA

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Crystallins, the major structural proteins in the lens, are also expressed outside the lens including the retina (Magabo et al., 2000; Xi et al., 2003) and are increased in the retina from diabetic rats (Heise et al., 2013), in response to intense light (Organisciak et al., 2006), and in drusen of AMD patients (Crabb

et al., 2002). The objective of this study was to determine if an external stress, UVA-irradiation, caused increased extralenticular expression of crystallins in the retina. The rat was chosen because of documented expression of crystallins in the rat retina (Kapphahn et al., 2003). Furthermore, the rat lens absorbs very little radiation at 355 nm and maximum effect will be on the retina (Dillon et al., 1999). Our data supported this, since no damage was apparent to the lens upon visual inspection and analysis by two-dimensional electrophoresis (2DE). A protocol for animal handling was developed with the Oregon Health and Science University Veterinarian staff and approved by the institutional committee. Briefly, 8-week-old Sprague-Dawley rats were fed basal rodent diet. Animals were subjected to general anesthesia for the procedure during which basic sensory responses were monitored. Eyes were treated with a 355 nm laser. Each eye was exposed to 2 minutes at 15 mJ per second and 25 J/cm². Animals were allowed to recover for 1 week before necropsy and dissection of the retina. Retinas were processed and analyzed by 2DE and then visualized by immuno-blotting with antibody made against rat alpha- or beta-crystallins. Our data showed increased expression of the crystallins in the retina in response to UVA treatment. Associated with this increase is an increase in acidic species of crystallins. We hypothesize that crystallins, especially the alpha-crystallin chaperones, may play a protective role in normal retina. However, overexpression of crystallins may also be pathogenic.

IRB Status: Approved

Disclosures:

KIRSTEN LAMPI: No financial relationships to disclose

ALPHA-A-CRYSTALLIN PREVENTS LENS EPITHELIAL CELL APOPTOSIS THROUGH NEGATIVE REGULATION OF p53-MEDIATED SIGNALING PATHWAY

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Purpose: Cataract is a major ocular disease that causes blindness in many developing countries. Studies from our laboratory and many others have shown that apoptosis plays a critical role in both non-congenital and congenital cataractogenesis. AlphaA-crystallin, a lens structure protein and a small heat shock protein, is a major guardian factor against stress-induced apoptosis. Mice deficient in alphaA (alphaA^{-/-}) display significant apoptosis in lens epithelium followed by formation of obvious cataracts. The underlying molecular mechanism against apoptosis by alphaA-crystallin is largely unknown. This study investigates the possible anti-apoptotic mechanism of alphaA through regulating the p53-mediated apoptotic signaling pathway.

Methods: MTT assays and cell flow cytometry were used to measure cell viability under different conditions. Co-immunoprecipitation assays were used to investigate interactions between alphaA and p53. Reverse transcription polymerase chain reaction and Western-blot analysis were utilized to study the

regulation of p53 signaling pathway in human lens epithelial cells (HLECs).

Results: Our results showed that alphaA can directly bind to p53. Such interaction lead to significantly reduction of p53 level and activity. In addition, alphaA also attenuates activation of the upstream kinase, ATM, leading to down-regulation of p53 activity. As a result, expression of its downstream target genes such as Bax was also downregulated. Mechanistically, alphaA enhances the interaction between p53 and its ubiquitin E3 ligase to promote p53 degradation.

Conclusions: Our observations demonstrate that alphaA can protect apoptosis and cataractogenesis through negative regulation of p53-mediated signaling pathway.

IRB Status: None

Disclosures:

DAVID LI: No financial relationships to disclose

P149

CHARACTERIZATION OF THE V41M MUTANT OF HUMAN GAMMA S CRYSTALLIN – HOW IS IT CATARACTOGENIC?

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GammaS crystallin does not show liquid-liquid phase separation, and attempts to crystallize it have been unsuccessful. Aggregation of other crystallins is ameliorated in the presence of gammaS crystallin. Here we examine how the properties of human gammaS crystallin (HGS) are affected when HGS undergoes a cataract-associated mutation. To address this question, we have characterized the V41M mutant of HGS, which is associated with congenital cataract. We have already shown specific structural changes that occur in the position/orientation of the two greek-key motifs in the N-terminal domain of the mutant that are transmitted further to the strands in the C-terminal domain, close to the domain interface. These changes lead to an increase in surface hydrophobicity in the mutant relative to the wild-type, as revealed by the binding of 8-Anilinonaphthalene-1-sulfonate (ANS). We have docked two molecules of ANS on a structural model of V41M, to account for the residue-specific information provided by the NMR studies on ANS binding. Using static and dynamic light scattering, we observe that the mutant readily forms non-reducible (i.e. non disulfide-linked) aggregates that are an order of magnitude larger in size, under conditions at which the wild-type is largely monomeric. An analysis of changes in the second virial coefficient (B₂₂), obtained using static light scattering, in terms of altered interactions in V41M shows that while HGS and V41M both exhibit overall attractive interaction, the magnitude of B₂₂ is lower for V41M. Further work is in progress to substantiate the structural and thermodynamic properties of HGS and V41M to fully understand how the V41M mutation leads to lens opacity.

IRB Status: Verified

Disclosures:

AJAY PANDE: No financial relationships to disclose

P150

THE OCULAR LENS AND ENVIRONMENTAL INSULT: INTERESTING NOVEL OBSERVATIONS

SHIWANI SHARMA¹, Sarah Martin¹, Alpna Dave¹, Mark Corbett³, Maurizio Ronci², Kathryn Burdon¹, Nicolas Voelcker², Jamie Craig¹

FLINDERS UNIVERSITY, OPHTHALMOLOGY¹; UNIVERSITY OF SOUTH AUSTRALIA, MAWSON INSTITUTE²; UNIVERSITY OF ADELAIDE, NEUROGENETICS RESEARCH PROGRAM³

The ocular lens because of its location receives external environmental insults. It is affected in both cataract and Pseudoexfoliation syndrome (PEX). External environmental factors contribute to both these diseases. We are interested in understanding the effect of environmental factors on the lens in these diseases. To determine distribution of proteins on the lens capsule from cataract and PEX patients, we performed matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) on specimens obtained from cataract surgery. MALDI-MSI showed that proteins, including extracellular matrix proteins, are concentrically and differentially distributed on the surface of the anterior lens capsule in cataract and PEX patients. The distribution pattern corresponds to the pupillary and parapupillary regions. We hypothesise that chronic environmental insults such as, ultraviolet radiation reaching the ocular lens may contribute to this distribution. To determine gene expression changes in lens epithelial cells in response to chronic dose of ultraviolet radiation, we performed microarray gene expression analysis in treated and untreated SRA01/04 human lens epithelial cells. The analysis revealed differentially expressed genes in an early response to ultraviolet radiation. The majority of the differentially expressed genes were down-regulated. These include nuclear transcription factors and genes directly or indirectly involved in regulating extracellular matrix proteins and cell adhesion/cell migration. These results support the above hypothesis. To determine the effect of chronic exposure to low doses of ultraviolet radiation on the lens *in vivo*, we performed experiments in mice. The employed doses (0.05-0.0125 J/cm²) induced cataract formation and the phenotype depended upon the dose of ultraviolet radiation used. Together, these studies indicate that environmental insults can have various effects on the ocular lens and affect its transparency.

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IRB Status: International

Disclosures:

SHIWANI SHARMA: No financial relationships to disclose

P151

PENETRATION OF TOPICALLY APPLIED CAFFEINE TO THE LENS AND BLOOD CIRCULATION

PER SÖDERBERG¹, Erik Forsman², Zhaohua Yu¹, Nooshin Talebizadeh¹, Stefan Löfgren³, Jonas Bergquist², Martin Kronschlager¹

GULLSTRAND LAB OF OPHTHALMOLOGY, UPPSALA UNIVERSITY, NEUROSCIENCE¹; UPPSALA UNIVERSITY, ANALYTICAL CHEMISTRY, DEPARTMENT OF CHEMISTRY-BMC²; KAROLINSKA INSTITUTE, ST. ERIKS EYE HOSPITAL³

Topically applied caffeine has been proven to decrease in vivo oxidative insult of ultraviolet radiation (UVR). The purpose of the current study was to qualitatively evaluate if 72 mM in vivo topically applied caffeine to the rat eye is toxic, the penetration of caffeine to the lens and blood as a function of time (30-120 min) after in vivo topical application of 72 mM to the rat eye, and the penetration of in vivo topically applied caffeine to the blood and lens as a function of concentration of caffeine applied to the rat eye (0-72 mM). The experimental animal was 150 g Sprague-Dawley female rats. Caffeine was dissolved in 0.9 % aqueous solution of hydroxypropylmethyl cellulose for topical application. Caffeine concentration in the lens was measured in the supernatant with HPLC after homogenization in pure water and centrifugation. Protein concentration in the lens was measured with the bicinchoninic acid (BCA) method. Lens caffeine was recorded as caffeine per mg of protein. In blood the caffeine concentration was measured directly in the supernatant after centrifugation. No toxic effects were observed macroscopically or in the slit lamp microscope after topical application of 72 mM caffeine. The concentration of caffeine dropped almost linearly in the lens and increased almost linearly in the blood in the time window studied. The concentration of caffeine increased almost linearly in the lens and in the blood, in the interval of topically applied caffeine concentration studied. It is concluded that topically applied caffeine dissolved in hydroxypropylmethyl cellulose penetrates dose dependently into the lens and blood.

IRB Status: Approved

Disclosures:

PER SÖDERBERG: No financial relationships to disclose

P152

LENS STIFFNESS IN CONNEXIN MUTANT LENSES

WIKTOR STOPKA, Eddie Wang, Tom Libby, Chun-Hong Xia, Xiaohua Gong, Hong Ma

UNIVERSITY OF CALIFORNIA, BERKELEY

Age-related increase in lens stiffness may be one contributing factor in the progression of presbyopia and nuclear cataract. We have been investigating the association between lens stiffness and nuclear cataract in connexin mutation lenses. A modified muscle lever system has been established to measure the stiffness of mouse lenses from wild-type (WT) and connexin mutant mice. Enucleated mouse lenses were carefully transferred to a DMEM-filled chamber, and compressed using a flat surface attached to the muscle lever. Lenses were compressed to a maximum of 2mN of force over 2 minutes while displacement values of the lever were recorded. A portable USB microscope camera was used to record the compression process. Displacement values at the point of maximum force were used in comparing the stiffness of lenses of differing age and genotype. Lenses of C57BL/6J (B6) WT, 129 WT, and Gja3 connexin

knockout (a3^{-/-}) mice in both strain backgrounds, between 4 weeks and 48 weeks of age, were used for comparable studies. Results show that 48-week old 129 WT lenses were compressed to 265 microns while 129 a3^{-/-} lenses were compressed to 155 microns at 2mN of force. Thus, the a3^{-/-} lenses appear stiffer than wild-type lenses in the 129 mouse strain background. However, both B6 WT lenses and B6 a3^{-/-} lenses show about 170-180um displacements at 2mN of force. Therefore, the lens stiffness is affected by different strain backgrounds and Gja3 gene knockout. Severe nuclear cataract of 129 a3^{-/-} seems associated with an increase of lens stiffness. We are in the process of measuring both wild-type and a3^{-/-} lenses from different strain backgrounds at younger ages. This modified device provides a good indication of stiffness with the potential for further analysis of other mechanical properties of the lens.

Supported by grants EY013849 from the National Eye Institute

IRB Status: None

Disclosures:

WIKTOR STOPKA: No financial relationships to disclose

P153

CASPASE-3 IN ULTRAVIOLET RADIATION CATARACT

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UPPSALA UNIVERSITY, GULLSTRAND LAB OF OPHTHALMOLOGY, DEPARTMENT OF NEUROSCIENCE

The purpose of this study was to determine the evolution of active caspase-3 expression in the normal lens and in the lens exposed to low dose UVR-300nm. Forty Sprague-Dawley rats were unilaterally exposed in vivo to 1 kJ/m² UVR-300nm for 15 minutes. At 0.5, 8, 16, and 24 hours after the exposure, both lenses were removed and processed for immunohistochemistry. The number and distribution of the cells labeled for active caspase-3 in the exposed and non-exposed lenses were compared among the four different time points. In normal lenses, active caspase-3 expression was abundant in the anterior pole of the lens. The probability of a radial spatial distribution of the labelling was fitted to a logistic model. The average number of cells in each half of the lens epithelium was estimated to be 213 cells. The increased rate of active caspase-3 expression was at 23±3 cell-1 and the inflection point was estimated at cell number 114±3 (0.95%CI). Comparing the exposed to the non-exposed lenses showed that the expression of active caspase-3 was higher in the exposed lenses (95%CI=0.18±0.02). The mean differences between the exposed and non-exposed lenses were 0.17±0.02, 0.20±0.03, 0.21±0.03, and 0.11±0.04 (95%CI) for the 0.5, 8, 16, and 24-hour time groups, respectively. There were no differences in the expression of caspase-3 between the 0.5 and 24-hour groups or between the 8 and 16-hour groups (95%CI=0.02±0.03). An orthogonal comparison showed a difference when comparing the 0.5 and 24-hours groups to the 8 and 16-hours groups (95%CI=0.06±0.03). It is concluded that Active caspase-3 is present in normal lens epithelial cells, with active caspase-3 expressed more at the anterior pole of the lens and decreasing towards the periphery. After the UVR exposure, the

expression of active caspase-3 in the lens increased, with peak expression detected approximately 16 hours after the exposure.

IRB Status: International

Disclosures:

NOOSHIN TALEBIZADEH: No financial relationships to disclose

P154

CONTROL OF LENS FIBER DEVELOPMENT BY LHX2-REGULATED NEURORETINAL FGFs

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NEUROSCIENCE

Fibroblast growth factor (FGF) signaling is an essential regulator of lens epithelial cell proliferation and fiber cell differentiation. However, the identities of these FGF factors, their source tissue, and the genes regulating their synthesis are not well-characterized. We have found that Chx10-Cre; Lhx2lox/lox mice, which selectively lack Lhx2 expression in neuroretina beginning at E10.5, show an early arrest in lens fiber development along with severe microphthalmia and retinal disorganization. These mutants show reduced expression of multiple neuroretinal-expressed FGFs in neuroretina, and likewise show reduced expression of canonical FGF target genes in both the neuroretina and lens. When FGF expression was selectively restored in Lhx2-deficient neuroretina using a CAG-lox-stop-lox-Fgf10 transgene, we observed a dramatic rescue of the defects in lens but not in neuroretinal development. These data demonstrate that neuroretinal-derived FGF factors are both necessary and sufficient to drive lens fiber development *in vivo*.

IRB Status: Approved

Disclosures:

THUZAR THEIN: No financial relationships to disclose

P155

STATISTICAL-THERMODYNAMIC MODEL FOR NONMONOTONIC DEPENDENCE OF LENS PROTEIN LIGHT SCATTERING AND PHASE BOUNDARIES ON MOLECULAR INTERACTIONS

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ROCHESTER INSTITUTE OF TECHNOLOGY, PHYSICS¹; ROCHESTER INSTITUTE OF TECHNOLOGY, MATHEMATICS²

Evidence for non-monotonic dependence of the light scattering and phase boundaries of concentrated mixtures of gamma and alpha crystallins on the strength of their intermolecular attractions has been provided by light scattering, small-angle neutron scattering, molecular simulations, and statistical-thermodynamic perturbation theory applied to bovine gamma/alpha mixtures, and by studies of mixtures of alpha with a cataractogenic mutant of human gammaD-crystallin. We show how such non-monotonic dependence can emerge as a general

feature of the intensive, multicomponent Gibbs free energy of concentrated mixtures, through analyzing the map of the ternary composition triangle into the space of the second partial composition derivatives of the free energy. We then apply this analysis to a statistical-thermodynamic, sticky-sphere model for lens protein mixtures that quantitatively reproduces measured absolute light scattering intensities of alpha/gamma mixtures. The analysis of this tested model confirms that small increases or decreases in the strength of alpha/gamma attractions, of size comparable to kT , are both capable of leading to greatly increased light scattering in realistically concentrated alpha/gamma mixtures. In the context of the model we identify solution and protein features that contribute to this sensitivity of light scattering to interspecies attraction. In particular, the model illustrates how weak alpha/gamma attractions can effectively decouple light scattering from the prominent local composition fluctuations in a large part of the composition triangle, promoting transparency. In contrast, even weaker alpha/gamma attractions promote compositional phase separation, leading to opacification, while stronger attractions couple light scattering and fluctuations and thus lead to increased light scattering by a different mechanism.

IRB Status: None

Disclosures:

GEORGE THURSTON: No financial relationships to disclose

P156

MODULATION OF THE PHOSPHORYLATION STATUS OF THE $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ COTRANSPORTER IN THE BOVINE LENS

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NEW ZEALAND NATIONAL EYE CENTRE, UNIVERSITY OF AUCKLAND, SCHOOL OF MEDICAL SCIENCES¹; UNIVERSITY OF AUCKLAND²

The $\text{Na}^+/\text{K}^+/\text{Cl}^-$ Cotransporter (NKCC1) has been implicated in cell volume regulation, ion transport and cell growth processes, all of which are crucial for normal lens physiology and transparency. NKCC1 has been identified at the RNA and protein level in the rat (1) and human lenses (2). Incubation of lenses in the presence of the NKCC1 inhibitor, bumetanide, inhibits 86Rb^+ fluxes in the rabbit lens (3, 4), and induces tissue damage in the rat lens (1), suggesting that ion fluxes mediated by NKCC1 are constitutively active in the lens. NKCC1 activity in other tissues is up-regulated by phosphorylation of the transporter. In this study we have performed Western blotting using two antibodies that detect either the phosphorylated or non-phosphorylated form of NKCC1 (5) to measure the effect of incubating bovine lenses in different osmolarities on the phosphorylation status of NKCC1. Our results show NKCC1 phosphorylation is increased by hyperosmotic challenge in a time and dose-dependent manner, and indicate that modulation of NKCC1 activity is important for the maintenance of lens cell volume. Furthermore, the NKCC1 phospho-signal could be manipulated under isotonic conditions by the addition of factors that interfere with the activity of the phospho-signaling system of NKCC1, including factors previously shown to stimulate fibre cell elongation. These results show that a vari-

ety of stimuli can dynamically regulate NKCC1 phosphorylation and suggest that changes in transporter activity can be used to either modulate fibre cell volume or drive fibre cell elongation.

This work was supported by the HRC of NZ and the Auckland Medical Research Foundation.1. Chee et al., *Mol Vis* 16:800-812, 2010.2. Wang et al., *IOVS*, 54:1135-1143, 2013.3. Alvarez et al., *Exp Eye Res*, 76:61-70, 2003.4. Alvarez et al., *Exp Eye Res*, 73:669-680, 2001.5. Flemmer et al., *J Biol Chem*, 277:37551-37558, 2002.

IRB Status: International

Disclosures:

IRENE VORONTSOVA: No financial relationships to disclose

P157

ESTABLISHING A MODEL TO INVESTIGATE ANTERIOR EPITHELIAL CELL DIVISION IN WHOLE PIG LENSES

REBECCA ZOLTOSKI¹, Taia Cordel¹, Richard Miller¹, Steven Quan¹, Andrew Ritter¹, George McArdle²

ILLINOIS COLLEGE OF OPTOMETRY¹; LENTICULAR RESEARCH GROUP, LLC²

Background and aims: We are currently investigating methods to establish the location and mitotic rate of germinative zone lens epithelium (GZ LE) in whole pig lenses. We are establishing a technique to identify GZ LE cells that are in the mitotic state by applying the DNA marker, 5-ethynyl-2'-deoxyuridine (EdU). Establishing mitotic cell division rates will ultimately

help assess changes due to laser ablation targeting these cells as a potential treatment for presbyopia.

Methods: Fresh pig eyes were obtained from a local abattoir. Following dissection, whole lenses were warmed, and then treated with chymotrypsin to remove ciliary body and then exposed to 0.5 mM EdU for 2 hours. Samples were fixed and permeabilized, and then EdU was visualized using Click-iT Alexa 594 fluorescence replication marker. Samples were counterstained with Hoechst 3324 to identify cell nuclei. Controls with no EdU were analyzed to assess specificity. The whole lenses were secured on a stage so that the anterior surface was flat and pictures were obtained using a Nikon AR1 multiphoton microscope.

Results: The GZ of the pig lens appears to be located approximately 500 µm from the lens equator in a band that is about 500 µm wide. Approximately 0.5% of GZ LE cells were labeled with EdU. Changes in the methods to increase specific EdU uptake have included warming the samples in media once dissected and increasing the concentration and time of EdU incubation. So far, none of these have resulted in significant changes in EdU labeling.

Conclusions: This method will allow us to establish germinative zone location and size in a whole lens, which ensures accurate location and size determinations. These methods will assist our lab in our studies utilizing laser ablation of GZ LE.

IRB Status: None

Disclosures:

REBECCA ZOLTOSKI: No financial relationships to disclose

Posterior Segment

Viewing: 10:00 – 10:30
11:45 – 13:00

Session with Authors: 15:00 – 16:30

P201**SIMVASTATINS INHIBIT PATHOLOGICAL RETINAL ANGIOGENESIS IN VLDLR MOUSE MODEL**

SABU ABRAHAM, John Greenwood, Steven Moss,
Xiaomeng Wang

UCL INSTITUTE OF OPHTHALMOLOGY, DEPARTMENT OF CELL
BIOLOGY

Statins (HMGCoA reductase inhibitors) have been widely used in the clinic as cholesterol lowering drugs in cardiovascular diseases. Recent reports have shown that, depending on context, statins may have either pro- or anti-angiogenic properties. In this study we investigated the effect of simvastatin administration in mouse models of ocular angiogenesis. The effect of simvastatin administration on ocular angiogenesis in oxygen induced retinopathy (OIR) and the very low density lipoprotein receptor (VLDLR) knockout mouse was evaluated. Consistent with previous studies¹, daily intra-peritoneal administration of simvastatin in OIR at a concentration of 1mg/kg of body weight did not cause a significant difference in neovascular tuft formation. In the VLDLR knockout mouse however, where mutations in VLDLR exhibit retinal neovascularisation as well as choroidal anastomosis², IP injection of simvastatin at 1mg/kg body weight (from p12 for 10 days) resulted in a significant reduction in intraretinal neovascularisation. This study demonstrates the context-dependent nature of statin therapy in the treatment of ocular neovascular complications.

Medina et al; *PlusOne* 3:7, 2008

Heckenlively et al; *Retina* 23:518–522, 2003

IRB Status: None

Disclosures:

SABU ABRAHAM: No financial relationships to disclose

P202**INTERACTIVE REGULATORY NETWORKS IN RETINAL ISCHEMIA-REPERFUSION INJURY**

KALINA ANDREEVA, Maha Soliman, Nigel Cooper

UNIVERSITY OF LOUISVILLE

The health and function of the retina relies on a collaborative interaction between diverse classes of molecular regulators

such as microRNAs (miRs) and transcription factors (TFs). Studies propose that miRs target predominantly TFs rather than other types of protein coding genes and suggest a possible interconnection of these two regulators in co-regulatory networks. Our lab has generated mRNA and miRNA microarray expression data to investigate time-dependent changes in gene expression, following induction of ischemia-reperfusion (IR) injury in the rat retina. Data from three reperfusion time points following retinal IR-injury (0h, 24h and 7d) were analyzed. Paired expression data of miRNAs–mRNAs were used to identify regulatory loops whose expression was altered by the IR injury paradigm. These loops were further integrated into larger regulatory sub-networks. In our preliminary results three sub-networks corresponding to 24h time point and two sub-networks corresponding to 7d time point were generated. The top molecular and cellular functions for the networks at 24h were cell death, cell survival and ion transport, while activation of complement system was the top function for each of the networks corresponding to the 7d time point. According to these results it is plausible to propose that different circuits might be executing different functions at different time periods, perhaps in different cell types, after IR injury. The purpose of our ongoing study is to identify ischemia-relevant and/or time point-specific “signature-modules”, the manipulation of which might influence the development and progression of retinal ischemic-related diseases. Further analyses to experimentally validate some of these regulatory modules and identify the biological significance of their coordinated regulation in healthy and diseased retina are in progress.

IRB Status: None

Disclosures:

KALINA ANDREEVA: No financial relationships to disclose

P203**EXPRESSION OF INTERCELLULAR ADHESION MOLECULE-1 BY HUMAN RETINAL ENDOTHELIAL CELLS IN RESPONSE TO INFLAMMATORY STIMULI**

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FLINDERS UNIVERSITY

Intercellular adhesion molecule (ICAM)-1 is an immunoglobulin superfamily member that plays a major role in physiological and pathological leukocyte trafficking across the vascular endothelium from blood stream to tissue. Retinal endothelial ICAM-1 may mediate migration of multiple leukocyte subsets into the retina in different forms of human posterior uveitis. Targeting ICAM-1 expression is a potential therapeutic approach for this disease. However, the ICAM-1 promoter contains multiple cis-regulatory motifs, and retinal endothelial expression is likely to depend on specific stimulus. We investigated the expression of ICAM-1 by human retinal endothelial

cells in response to different inflammatory stimuli. Confluent monolayers of a human retinal endothelial cell line (Bharadwaj and Appukuttan et al, *Prog Retin Eye Res* 2013; 32:102-80) were treated with lipopolysaccharide (LPS, 10 microg/ml), tumor necrosis factor (TNF)-alpha (10 ng/ml), interferon (IFN)-gamma (10 ng/ml), vascular endothelial growth factor (VEGF165, 20 ng/ml), interleukin (IL)-1beta (5 ng/ml), IL-6 (100 ng/ml), IL-17A (10 and 100 ng/ml) and IL-22 (10 and 100 ng/ml) for 2, 6, 24 and 48 hours. Total RNA was isolated and reverse transcribed, and relative expression of ICAM-1 transcript, normalized to 18S rRNA, was determined by quantitative real-time PCR. Retinal endothelial ICAM-1 was significantly ($p < 0.05$) increased across the studied intervals by LPS (≥ 5.8 -fold), TNF-alpha (≥ 17.7 -fold) and IL-1beta (≥ 4.3 -fold). IFN-gamma and IL-6 induced significant ICAM-1 expression at early time points (IFN-gamma: 2.5-fold at 2 hours; IL-6: 2.9-fold at 6 hours) and VEGF165 induced expression at 48 hours (1.8-fold). IL-17A and IL-22 did not increase ICAM-1 expression at the concentrations and time points tested. These observations indicate ICAM-1 is differentially induced in human retinal endothelial cells by different inflammatory stimuli. Future studies will address the transcriptional processes within human retinal endothelial cells that control ICAM-1 expression under different inflammatory conditions.

IRB Status: None

Disclosures:

BINOY APPUKUTTAN: No financial relationships to disclose

P204

PGRMC1, ALSO KNOWN AS SIGMA RECEPTOR 2, IS A CRITICAL REGULATOR OF IRON HOMEOSTASIS IN THE RETINA

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Iron is an essential component of important biological processes; however, accumulation of excessive iron in cells is toxic, leading to cell death via oxidative stress. This provides the logic for the involvement of multiple proteins in iron homeostasis. Disruptions in the functions of these proteins lead to either iron overload with resultant hemochromatosis or iron deficiency with resultant anemia. S2R (sigma-2 receptor)/PGRMC1 (progesterone receptor membrane component 1) is a heme-binding protein, and has a role in steroid metabolism, reproduction, and cancer. Recent studies have shown that Dap1, a yeast homolog of PGRMC1, is involved in iron homeostasis. However, to date there is no evidence for such a role for PGRMC1 in mammalian cells. Here we demonstrate that PGRMC1 is a key determinant of iron homeostasis in the retina. Expression of PGRMC1 was examined in wild type and *Hfe*^{-/-}, *Hju*^{-/-} (Iron overload) and *mask*^{-/-} (Iron deficiency) mouse retinas and in primary retinal pigment epithelial (pRPE) cells isolated from these mice. qPCR and immunofluorescence techniques were used to assess PGRMC1 expression. PGRMC1 is expressed in all retinal cells including ganglion cells, Müller cells, photoreceptor cells and

RPE. The expression is comparable in RPE and Müller cells but higher in ganglion cells. The PGRMC1 expression is increased in the retinas of *Hfe*^{-/-} and *Hju*^{-/-} mice but decreased in the retinas of the iron-deficient *mask*^{-/-} mice. The upregulation of PGRMC1 expression by excessive iron is also evident in pRPE cells isolated from *Hfe*^{-/-} and *Hju*^{-/-} mice. Furthermore, treatment of ARPE-19 cells with an inhibitor of PGRMC1 suppresses the expression of HFE and HJV. In vitro studies with pRPE cells show that excessive iron increases PGRMC1 expression. These studies unveil a novel, hitherto unrecognized, function of S2R/PGRMC1 in mammalian cells as a critical regulator of iron homeostasis.

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IRB Status: Verified

Disclosures:

PACHIAPPAN ARJUNAN: No financial relationships to disclose

P205

CELLULAR THERAPY WITH KAINATIS OPTICONEUROPATHY

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Purpose: To research the effectiveness of embryonal stem cells transplantation on manifestation of toxic opticopathy.

Methods: The rabbits with weight 3,0 – 3,5 kg, acid kainatis 0,04 mg (Sigma, USA). Embryonal stem cells from mouse male's embryo ER 51, visualization was realized with the help of analyzer « RETCAM ». The solution of acid kainatis 0,04 mg was input in rabbits into the vitreous body for formation of opticoneuropathy on the 7th day. The 1st group: the input of acid kainatis intravitreous 0,04 mg. The 2nd group: input in region retrobulbar of embryonal stem cells for formation kainatis opticoneuropathy. The inspections were carried out in dynamics.

Results: The evaluation of use effectiveness of these preparations is: the disappearance of haemorrhage on the nerve visual papillae, the beginning for collapse of retina's ganglionic cells axons, the full collapse of fiber, atrophy of the nerve visual papillae. Morphological researches have shown that at introduction embryonal stem cells cages at rabbits with kainatis opticoneuropathy the layer neuronal fibres caudal department of a disk of an optic nerve (450 days of supervision) in comparison with control group kainatis opticoneuropathy without introduction embryonal stem cells (a full atrophy neuronal fibres for 160 days) remains.

Conclusions: Input in vitreous body of acid kainatis in quantity if 0,04 mg induces the development kainatis opticoneuropathy. Input embryonal stem cells in region retrobulbar rabbits is accompanied with expressive slowing down of atrophy of visual nerve and ganglionic cells axons, which certificates about a high neuroprotective action of stem cells.

IRB Status: None

Disclosures:

PAVEL BELIAKOUSKI: No financial relationships to disclose

RETINAL UPREGULATION OF PRO-INFLAMMATORY MEDIATORS AND GROWTH FACTORS IN HIGH FAT DIET FED TYPE 2 DIABETIC MICE

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Diabetes causes vascular inflammation in the retina and breakdown of the blood retinal-barrier (BRB), resulting in macular edema, a major cause of visual loss. Increased BRB permeability is associated with changes in the expression of vascular endothelial growth factor (VEGF) and of many pro-inflammatory mediators. We have previously shown that these changes could be reversed by altering the kinin pathway through B1 receptor antagonism in streptozotocin-diabetic rats. The present study aims at determining the beneficial effects of a 2-week treatment with a B1R antagonist (SSR240612; 10 µg/g/d s.c.) given alone or together with a standard antidiabetic agent, thiazolidinedione (TZD, a PPARγ agonist; 1.6 µg/g/d s.c.) on retinal vascular gene expression in a nutritional model of type 2 diabetes. Comparison was made between mice treated with a high fat diet (HFD) (diabetic) and a standard diet (SD) (control) for 20 weeks. The qRT-PCR analysis showed an overexpression of kinin receptors (B1R and B2R), growth factors (VEGFA and VEGFR-2) and inflammatory targets (iNOS, eNOS, NF-κB) in the retina of HFD-diabetic mice in comparison with control mice. Immunohistochemistry also revealed increased immunoreactivity of VEGF in diabetic retina. A significant reduction in the expression of VEGFA and VEGFR2 was observed with SSR240612 as compared with TZD, which was correlated with the lack of VEGF staining in SSR240612 group but not in TZD group. The mRNA expression of B1R and B2R was reduced by SSR240612 and further blunted if combined with TZD treatment in diabetic mice, yet TZD treatment alone had no impact. Whereas iNOS expression was notably reduced by SSR240612 given alone, eNOS and NF-κB mRNA levels were not affected by any treatments. These data suggest that the simultaneous inhibition of kinin B1R and activation of PPARγ could be of therapeutic value in reducing vascular inflammation in diabetic retinopathy.

IRB Status: None

Disclosures:

MENAKSHI BHAT: No financial relationships to disclose

NEURITE REGENERATION IN ADULT RAT RETINAS EXPOSED TO LOW DOSE ADVANCED GLYCATION END-PRODUCTS AND REGENERATIVE EFFECTS OF NEUROTROPHIN-4

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The purpose of this study was to determine the effect of AGEs on neurite regeneration and regenerative effects of neurotrophin-4. Retinal explants of 4 adult rats were cultured on

collagen gel and were incubated in (1) serum-free control culture media, (2) 10 µg/ml glucose-AGE-BSA, (3) 10 µg/ml glycolaldehyde-AGE-BSA, (4) 10 µg/ml glyceraldehyde-AGE-BSA, (5) 10 µg/ml glucose-AGE+100ng/ml neurotrophin-4, (6) 10 µg/ml glycolaldehyde-AGE-BSA+ 100ng/ml neurotrophin-4, or (7) 10 µg/ml glyceraldehyde-AGE+ 100ng/ml neurotrophin-4, (8) 100ng/ml NT-4 supplemented media without AGE-BSA, or (9) 10 µg/ml glucose-AGE-BSA + 2 µM caspase-9 inhibitor supplemented culture media. After 7 days, the number of regenerating neurites with growth cones was counted, explants were fixed, cryosectioned, and stained for TUNEL. The ratio of TUNEL-positive cells to all cells in the ganglion cell layer was determined. Immunohistochemistry was performed to examine whether caspase-independent cell death factor, apoptosis inducing factor (AIF) and active-forms of caspase-9 expression were related to the neuronal cell death induced by AGE. All data was expressed as mean ± standard deviation (SD). Statistical analyses were carried by one-way ANOVA with Scheffe's F tests. Study showed that the AGEs are neurotoxic and inhibit regeneration of neuritis of retinal neurons even at low concentrations. High-dose AGEs significantly impede neurite regeneration independent of increasing neuronal cell death. The neurotoxic effect of AGEs is associated with increased expression of AIF and the active-form of caspase-9. There may be biochemical links between caspase-9 and AIF activation. NT-4 significantly enhances neurite regeneration in retinas exposed to AGEs. The neuroprotective and regenerative effects of NT-4 are correlated with the reduction of caspase-9 and AIF expression.

IRB Status: International

Disclosures:

GUZEL BIKBOVA: No financial relationships to disclose

DETECTION AND LOCALIZATION OF MICRORNA-34A BY IN SITU HYBRIDIZATION IN THE POSTERIOR POLE OF THE MOUSE EYE

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MicroRNAs are emerging as important post-transcriptional regulators of gene expression of many biological processes such as cell cycle control, apoptosis and aging, but not much is known about their distribution in the ocular tissue. The purpose of this study was to determine the localization of microRNA-34a expression in the posterior pole of the mouse eye using a novel technique of LNA in situ hybridization (ISH). Mouse eyes from C57BL/6 J strain were harvested from 4 different age groups: 4, 18, and 24 month. In situ hybridization was performed on 3 biological replicas for each time point, following manufacturer's protocol. Paraffin embedded mouse eyes were sectioned under RNase free conditions. Exiqon's LNA probes were used: negative control-scrambled probe, positive control-U6 and for detection of miR-34a we used miR-34a probe. RT-PCR was performed using ABI Taqman probes followed by analysis using SDS 2.4 software. MiR-34a labeling was highly visible in the inner segment (IS) of the rods and cones at all time points.

In addition, in the 18 and 24 month old mouse, the ganglion cell layer (GCL), inner nuclear layer (INL) and the outer nuclear layer (ONL) also demonstrated labeling with miR-34a probe. RT-PCR results on mouse retina and RPE/choroid confirmed these observations. Our results demonstrate that we can locate miR-34a in the mouse eye posterior pole using LNA-ISH approach. Older eyes showed an increase in miR-34a expression, demonstrated by the increased probe labeling by ISH, and verified with RT-PCR. Increase in miR-34a expression correlated with advancing age of the mouse eye.

IRB Status: Approved

Disclosures:

MATTHEW BORDBARI: No financial relationships to disclose

P209

NFATC1 AND NFATC2 DIFFERENTIALLY REGULATE TNF α - AND VEGF-INDUCED INFLAMMATION IN RETINAL MICROVASCULAR ENDOTHELIAL CELLS

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Retinal levels of vascular endothelial growth factor (VEGF) and tumor necrosis factor alpha (TNF α) are known to be elevated at early disease stages in diabetic patients. In retinal microvascular endothelial cells (RMEC), VEGF and TNF α induce production of inflammatory cytokines such as IL-6 and IL-8, which are known to be increased in the vitreous of diabetic patients and to contribute to diabetic retinopathy pathology. The nuclear factor of activated T-cells (NFAT) family of transcription factors upregulate expression of numerous inflammatory gene targets, and NFAT is known to act downstream of VEGF and TNF α in certain contexts. The present study aimed to evaluate the specific role of NFAT isoforms NFATc1 and NFATc2 under VEGF and TNF α treatment conditions in RMEC. Human RMEC were transfected with isoform-specific siRNA, and treated with either VEGF (50ng/ml) or TNF α (1ng/ml) for 12hrs. VEGF treatment significantly increased secreted levels of IL-6 and IL-8, which NFATc1 knockdown inhibited by 46% (p=0.0022) and 59% (p=0.0006), respectively. NFATc2 knockdown inhibited VEGF-induced cytokine production of both IL-6 and IL-8 by 33% (p=0.0141) and 39% (p=0.0077), respectively. NFATc1 knockdown did not affect TNF α -induced IL-6, but did inhibit TNF α -induced IL-8 by 68% (p=0.0068), while NFATc2 knockdown had no effect on TNF α -induced cytokine production. These findings show a clear yet complex role for individual NFAT isoforms in RMEC inflammation. Given the important pathological impact that inflammatory mediators can have in the diabetic retina, NFAT isoform signaling may be a valuable and uniquely specific target for diabetic retinopathy treatment.

IRB Status: None

Disclosures:

COLIN BRETZ: No financial relationships to disclose

P210

THE BERLIN FAT MOUSE: A NEW MODEL TO INVESTIGATE RETINAL DEGENERATION

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The Berlin Fat Mouse Inbred (BFMI) line was initially generated from an outbreed population that underwent multiple selection processes focused on high fat content to analyze genetic influence on obesity. An organ-wide phenotype screening revealed retinal abnormalities. The aim of this study was to characterize the retinal morphology and functionality of BFMI mice in detail. An intercross experiment using BFMI female and C57BL/6 (B6, wild type) male was performed to eliminate preexisting albinism. Hematoxylin-eosin staining and immunohistochemistry was accomplished on retinal cross sections. Functional examinations using in-vivo electroretinography (ERG) were performed at postnatal day (P) 14, 21 and 28. Littermates of the second filial generation presented a dysfunction of coordinated rhodopsin trafficking, depending on their BFMI genotype. Homozygous BFMI mice revealed considerable rhodopsin accumulations in the outer nuclear layer (ONL) in all cases. Heterozygous BFMI mice presented mild accumulations of rhodopsin in 25% of the cases, while wild type littermates showed an intact retinal morphology without rhodopsin abnormalities. In addition to rhodopsin accumulations the ONL thickness in homozygous BFMI declined by 29% compared to wild type mice at P28 (p < 0.005). ERGs of homozygous BFMI mice demonstrated reduced a-, b- and c-waves at P14, 21 and 28. Regarding body mass, homozygous BFMI mice gained 24% more weight until P28 compared to wild type littermates at the same age (p = 0.032). In summary, BFMI mice revealed defined characteristics of retinal degeneration including a rhodopsin trafficking dysfunction and ONL thinning accompanied by obesity. Genetic analyses are in process to identify underlying mutations in the heterogenic background of BFMI mice of which whole genome sequencing is available. Thereby, the BFMI line represents a naturally bred mouse model with a potentially new type of retinal degeneration, which might enlighten detailed pathomechanisms and contribute to therapeutic approaches.

IRB Status: Approved

Disclosures:

CLAUDIA BROCKMANN: No financial relationships to disclose

P211

PROMOTING BIOAVAILABILITY OF EPOXYEICOSATRIENOIC ACID INHIBITS TNF α -INDUCED RETINAL VASCULAR INFLAMMATION

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Cytochrome P450 epoxygenases convert arachidonic acid (AA) to epoxyeicosatrienoic acids (EETs). In endothelium, EETs promote angiogenesis, induce hyperpolarization, and inhibit inflammation. The soluble epoxide hydrolase (sEH) enzyme limits EET bioavailability by converting it to a less bioactive diol. The role of EETs in retinal inflammation, a hallmark of early non-proliferative diabetic retinopathy (NPDR), is unknown. The purpose of this study was to determine the effect of EETs on TNF α -induced inflammation in human retinal microvascular endothelial cells (HRMEC) and an animal model of retinal vascular inflammation. HRMEC were treated with TNF α (1.0ng/ml), and EET production was assessed by LC/MS-MS. To determine the role of EETs on TNF α -induced inflammation, HRMEC were treated with TNF α (0.2ng/ml) in the presence or absence of 11,12-EET (0.5 μ M) and an sEH inhibitor, AUDA (1.0 μ M), and *MCP-1*, *VCAM-1*, and *ICAM-1* expression were assessed by qRT-PCR. Next, HRMEC were treated with TNF α (1.0ng/ml) in the presence or absence of 11,12-EET (0.5 μ M) and AUDA (10 μ M) and leukocyte adherence was analyzed by parallel plate flow chamber (PPFC). C57BL/6 mice were injected intravitreally with TNF α (50ng/ml) and their retinas were collected to determine the EET-to-diol ratio. Additionally, mice were injected with TNF α (50ng/ml) in the presence or absence of 11,12-EET (0.5 μ M) and AUDA (10 μ M), and leukostasis was assessed by perfusion with Concanavalin-A. TNF α inhibited total HRMEC EET production by 52%. 11,12-EET and AUDA inhibited TNF α -induced *MCP-1* (31%), *VCAM-1* (62%) and *ICAM-1* (31%) expression. 11,12-EET and AUDA inhibited TNF α -induced leukocyte adherence by 49% in PPFC. *In vivo*, TNF α reduced the EET-to-diol ratio by 52% and 11,12-EET and AUDA inhibited TNF α -induced leukostasis by 44%. In this study, we demonstrated that TNF α treatment reduced HRMEC EET production, and that treatment with 11,12-EET and the sEH inhibitor, AUDA, reduced TNF α -induced retinal leukostasis. Future studies will determine the efficacy of EET treatment in rodent models of NPDR.

IRB Status: None

Disclosures:

MEGAN CAPOZZI: No financial relationships to disclose

P212

DAYLIGHT VISION REPAIR BY CELL TRANSPLANTATION

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Human daylight vision depends on cone photoreceptors and their degeneration results in visual impairment and blindness as observed in several eye diseases including age-related macular degeneration or late stage retinitis pigmentosa, with no available cure. Pre-clinical cell replacement approaches in mouse retina are focused on rod dystrophies, due to the availability of sufficient donor material from the rod-dominated mouse retina, leaving the development of treatment options for cone degenerations not well studied. Thus, an abundant and traceable source for donor cone-like photoreceptors was

generated by crossing the neural retina leucine zipper-deficient (*Nrl*^{-/-}) mice with an ubiquitous GFP reporter line. Cone-like photoreceptors were enriched by CD73-based magnetic associated cell sorting and transplanted into the subretinal space of adult wild-type, cone-only *Nrl*^{-/-} or cone degeneration (*Cpfl1*) mice. Donor cells correctly integrated into host retinas, acquired mature photoreceptor morphology, expressed cone-specific markers and survived for up to six months, with significantly increased integration rates in the cone-only *Nrl*^{-/-} retina. Integration rates were not influenced by subretinally located macrophages or monocytes. Individual retinal ganglion cell recordings demonstrated the restoration of photopic responses in cone degeneration mice following transplantation suggesting, for the first time, the feasibility of daylight vision repair by cell replacement in the adult mammalian retina.

IRB Status: None

Disclosures:

TIAGO FERREIRA: No financial relationships to disclose

P213

EFFECT OF GLUCOCORTICOIDS ON NEURONAL AND VASCULAR PATHOLOGY IN A TRANSGENIC MODEL OF SELECTIVE MÜLLER CELL ABLATION

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Retinal diseases such as macular telangiectasis type 2 (MacTel), age-related macular degeneration (AMD) and diabetic retinopathy (DR) affect both neurons and blood vessels. Treatments addressing both at the same time might have advantages over more specific approaches, such as vascular endothelial growth factor (VEGF) inhibitors which are used to treat vascular leak but are suspected to have a neurotoxic effect. Here, we studied the effects of an intravitreal injection of triamcinolone acetonide (TA) in a transgenic model in which patchy Müller cell ablation leads to photoreceptor degeneration, vascular leak and intraretinal neovascularization. TA was injected 4 days before Müller cell ablation. Changes in photoreceptors, microglia and Müller cells, retinal vasculature, differential expression of P75 neurotrophin receptor (p75NTR), tumor necrosis factor- α (TNF α), the precursor and mature forms of neurotrophin 3 (pro-NT3 and mature NT3) and activation of the p53 and p38 stress-activated protein kinase (p38/SAPK) signaling pathways were examined. We found that TA prevented photoreceptor degeneration and inhibited activation of microglial and Müller cells. TA attenuated Müller cell loss and inhibited overexpression of P75NTR, TNF α , pro-NT and the activation of p53 and p38/SAPK signaling pathways. TA not only prevented the development of retinal vascular lesions but also inhibited fluorescein leakage from established vascular lesions. TA inhibited overexpression of VEGF in transgenic mice but without affecting its basal expression in the normal retina. Our data suggest that glucocorticoid treatment may be beneficial for treatment of retinal diseases such as MacTel, AMD and DR that affect both neurons and the vasculature.

IRB Status: International

Disclosures:

MARK GILLIES: Consultant/Advisor relationship with Bayer; Consultant/Advisor relationship with Allergan; Consultant/Advisor relationship with Novartis

P214

ANTI-ANGIOGENIC EFFECT OF BAICALIN IN A MOUSE MODEL OF OXYGEN-INDUCED RETINOPATHY

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Baicalin (7-D-glucuronic acid, 5,6-dihydroxyflavone) is a flavonoid derived from the dried root of *Scutellaria baicalensis*. This study was performed to investigate whether baicalin is effective in inhibiting retinal neovascularization in an oxygen induced retinopathy (OIR) mouse model. ICR neonatal mice were exposed to 75% oxygen from postnatal day (P)7 until P12 and returned to room air (21% oxygen) for five days (P12 to P17). Mice were subjected to daily intraperitoneal injection of baicalin (1 mg/kg, 10 mg/kg) or saline from P12 to P17. Retro-orbital injection of FITC-dextran was performed and retinal flat mounts were viewed by fluorescence microscopy and photographed. Avascular area and neovascular tufts were quantified from the digital images in a masked fashion using image analysis software. Neovascular nuclei were also counted in the in OIR induced mouse (P17) and baicalin-injected mouse. Western blot was performed to characterize the angiogenic processes involved in OIR and to demonstrate the anti-angiogenic activity of baicalin in vivo. In the retina of baicalin-injected mouse, the central ischemic area was significantly decreased ($51.87 \pm 5.72\%$ vs $32.14 \pm 3.14\%$, $P < 0.01$). The mean numbers of neovascular nuclei counted in OIR induced mouse (P17) and baicalin-injected mouse were significantly different in HE staining. Hyperbaric oxygen exposure resulted in an increase of VEGF, PDGF and angiotensin and when mice were treated with baicalin, a dose dependent reduction in these proteins was observed. Our study demonstrates the anti-angiogenic effect of baicalin in a mouse model of OIR in vivo and our finding suggest that baicalin can be considered as one of the candidate supplementary substances to be used for the treatment of various ocular vascular pathologies.

IRB Status: Approved

Disclosures:

JAE WOOK HAN: No financial relationships to disclose

P215

INHIBITION OF α B CRYSTALLIN INDUCED MESENCHYMAL TO EPITHELIAL TRANSITION IN RPE CELLS

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Development of subretinal fibrotic scar formation can cause severe vision loss in age-related macular degeneration. An initial step of this process is epithelial-to-mesenchymal transition (EMT) of the retinal pigment epithelium (RPE). The role of α B crystallin, a prominent member of small heat shock protein family, in EMT has not been studied. The aim of this study was to investigate the role of α B crystallin in EMT of RPE cells. All studies were conducted in cultured human primary fetal RPE cells at two to four passages. We found the expressions of mRNA and protein of α B crystallin were not significantly altered in RPE cells after TGF β 2 stimulation. To examine whether modulation of α B crystallin expression can alter EMT markers, we investigated the effects of α B crystallin silencing on the expression of E-cadherin, α -SMA and the transcriptional factors such as Snail and Slug with or without TGF β 2 treatment by Western blotting and realtime RT-PCR. Suppression of α B crystallin by siRNA induced significant up-regulation of E-cadherin and down-regulation of α -SMA, Snail and Slug in mRNA and protein expressions ($p < 0.05$ vs controls). We observed that suppression of α B crystallin did not alter TGF β 2-induced phosphorylation of SMAD2/3 indicating that α B crystallin is likely not involved in TGF β /SMAD signaling pathway. We next tested the contribution of α B crystallin to fibrotic process such as proliferation and migration. Suppression of α B crystallin significantly reduced cell proliferation as determined by decreased BrdU incorporation ($p < 0.01$ vs controls) and inhibited cell migration in Oris cell migration assay ($p < 0.01$ vs controls). Our results show that α B crystallin plays a significant role in fibrotic process possibly through EMT regulation.

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IRB Status: None

Disclosures:

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P216

RETBINDIN IS A NOVEL PHOTORECEPTOR-SPECIFIC PROTEIN AND A MEMBER OF THE INTER-PHOTORECEPTOR MATRIX

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Retbindin is a novel retina-specific protein of unknown function. It has significant sequence homology to only one other protein: the riboflavin binding protein of chicken oviduct cells. To assess the importance of retbindin to the retina, we generated a knockout mouse (Retb^{-/-}), in which the retbindin coding sequence was replaced with that of eGFP. eGFP in Retb^{-/-} mice was found only in the photoreceptor cells, which was consistent with the distribution of retbindin in WT animals. Electroretinography revealed an age- and dose-dependent decline in both rod and cone responses at postnatal days (P)120 and 240. This functional decline is the result of both rod and cone photoreceptor cell loss as measured by outer nuclear layer counts and PNA stained flat mounts. Given the clear importance of

this protein for retinal structure and function, we further explored its properties and localization. Biochemical analysis of retbindin showed that it is secreted by rod photoreceptors and is maintained within the insoluble interphotoreceptor matrix. *In vitro* binding assays showed that retbindin's potential ligand is riboflavin. Immunofluorescence analysis localized retbindin to the outer segment/RPE interface. Retbindin's properties and localization suggest that it may play a role in retinal/RPE metabolite exchange and sequestration. Future research will be aimed at investigating its function *in vivo* to better understand the role of this novel protein in retinal homeostasis.

IRB Status: Approved

Disclosures:

RYAN KELLEY: No financial relationships to disclose

P217

GENOTYPE-PHENOTYPE ANALYSIS IN PATIENTS WITH AUTOSOMAL RECESSIVE RETINITIS PIGMENTOSA DUE TO PDE6A MUTATIONS

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The aim of the study was to perform genotype-phenotype correlations in patients with autosomal recessive retinitis pigmentosa (arRP) due to mutations in the PDE6A gene encoding the α -subunit of the rod cGMP-phosphodiesterase 6. Fifteen patients (9 females, 6 males; age: 18 to 78 years) with known mutations in the PDE6A gene were examined. Homozygosity was frequent and was observed in one sib pair carrying the p.V685M mutation, another sib pair and two unrelated patients carrying the p.R102S and the third sib pair and an unrelated patient carrying the p.R257* mutation. The remaining six patients carried compound heterozygous mutations (p.V685M/p.L621R; p.V685M/p.R102S; p.V685M/p.R562W; p.V685M/H226Tfs*2; p.R102S/p.H563Q; c.1926+1G>A (splice site mutation)/p.K21_Y23delinsN). The siblings homozygous for p.V685M showed markedly reduced visual function (mean VA: 0.05; mean VF for target III4e: 187.5 deg2) and no recordable electrophysiological responses. In comparison, the compound-heterozygotes revealed significantly better visual function (mean VA: 0.6; mean VF: 748 deg2). Although Ganzfeld ERGs were mostly extinguished, mfERGs could detect residual responses in these cases. The siblings homozygous for p.R102S presented with well-preserved function (mean VA: 1.0, mean VF: 10936.1 deg2) and remaining electrophysiological responses. The unrelated patients also showed a very mild progression even in later ages, but additional macular hole development was observed in one case. The patients homozygous for p.R257* presented with a well-preserved function, however, the two siblings suffered from additional macular edema. In conclusion, all patients with PDE6A mutations presented with typical features of arRP, however, depending on the genotype, severity of disease varied considerably. The p.V685M mutation seemed to cause worse clinical outcome — especially if patients

were homozygous for this mutation, while the p.R102S mutation was linked to milder disease manifestation. These findings will be useful for the identification of patients concerning future therapeutic trials.

IRB Status: Approved

Disclosures:

DITTA ZOBOR: No financial relationships to disclose

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ALDOSE REDUCTASE DEFICIENCY PROTECTS THE NEONATAL MOUSE RETINA AGAINST OXYGEN-INDUCED RETINOPATHY

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Retinopathy of prematurity (ROP) has become the leading cause of blindness and visual loss in children. Besides the well-known vascular abnormality, retinal dysfunction has also been reported even after ROP has resolved. We previously showed that genetic deletion of aldose reductase (AR), the rate-limiting enzyme in the polyol pathway, protected the neonatal vasculature and reduced neovascularization in the mouse model of oxygen-induced retinopathy (OIR), a well-established model of ROP. Here, we further investigated the effects of AR deficiency on retinal neuronal function and morphology. Seven-day-old wild-type (WT) and AR-deficient (AR^{-/-}) mouse pups were exposed to 75% oxygen for 5 days and then returned to room air. Electroretinography was used to examine the retinal function at P30. Retinal layer thickness was measured and compared. Various retinal neurons were identified by immunohistochemistry for calbindin (horizontal cell marker), PKC α (rod bipolar cell marker), calretinin (amacrine cell marker), and Tuj1 (retinal ganglion cell marker). Level of oxidative stress was assessed by immunohistochemistry for poly(ADP-ribose) (PAR). Our results showed that significantly reduced a-wave, b-wave and OPs were observed in WT but not in AR^{-/-} mice. While there was significantly reduced retinal inner nuclear layer and inner plexiform layer thickness in WT central retinae, AR^{-/-} retinae showed preserved inner nuclear layer and inner plexiform layer. Horizontal, rod bipolar and amacrine cells were partly protected by AR deficiency with an attenuated PAR immunoreactivity. Our results not only demonstrated the retinal neuronal changes in the mouse model of OIR, but also showed that these changes appeared to be prominent in central avascular area, indicating a link between vascular abnormality and neuronal changes. In addition, AR deficiency provides protection in retinal neurons possibly by reducing oxidative stress, suggesting a therapeutic potential of AR inhibition in the treatment of ROP with beneficial effects on retinal neurons.

IRB Status: None

Disclosures:

AMY LO: No financial relationships to disclose

DERIVATION AND DISEASE MODELING OF HUMAN PLURIPOTENT STEM CELL-DERIVED RETINAL GANGLION CELLS

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Derived from patient samples, human induced pluripotent stem cells (hiPSCs) have the potential to differentiate into any cell type of the body, providing a unique tool for cell replacement, disease modeling, and drug screening. To serve in this capacity, however, hiPSCs must be directed to properly differentiate to the cell type of interest. We have previously demonstrated the ability to differentiate hiPSCs to a retinal lineage, whereupon retinal photoreceptor and retinal pigment epithelium cells were the most abundant cell types produced. The ability to derive retinal ganglion cells (RGCs) from hiPSCs would not only serve as a novel model of human retinogenesis, but would also have profound implications for diseases such as glaucoma or other optic neuropathies. In the current study, we characterize the ability of hiPSCs to generate RGC phenotypes, including those cells expressing the RGC-specific transcription factors Brn3 and Math5. Furthermore, treatment of these cells with extrinsic factors known to influence the development of specific retinal cell types was demonstrated to affect the specification of RGCs from a more primitive retinal progenitor cell fate. More recent efforts have focused upon the establishment of lines of hiPSCs derived from patients with glaucoma, with the intent to develop an in vitro system with which to study inherent subcellular changes in glaucomatous neurodegeneration. The results of these studies allow for future use of these hiPSC-derived RGCs for studies of retinal development as well as neurodegenerative processes associated with optic neuropathies such as glaucoma.

IRB Status: Verified

Disclosures:

JASON MEYER: Patents/Royalties relationship with Wisconsin Alumni Research Foundation

MOLECULAR CHARACTERIZATION OF NOVEL COMPOUND HETEROZYGOUS MUTANTS ASSOCIATED WITH LEBER CONGENITAL AMAUROSIS IN CHINESE FAMILY

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Leber Congenital Amaurosis (LCA) is a hereditary early-onset retinal dystrophy. To elucidate the cause of one Chinese family with LCA, we performed a whole-exome analysis and identified a novel gene with compound heterozygous mutation. Identified gene belongs to a family of molecular

chaperones, which mutation in other family members are known to cause neuropathy. Additionally, it is known that one protein which interacts with this molecular chaperone evokes retinal dystrophy in experimental mouse model. Based on these backgrounds, we investigated the novel mutant at the molecular level. Whole-exome analysis was performed on 2 out of 6 siblings with LCA. Their unaffected family members were analyzed as control. Identified unrivalled candidate gene and its mutants were subjected to protein structure prediction. General molecular biological procedures were carried out. To elucidate the physiological impact, we monitored the dynamics of one protein known to be abundant in photoreceptor and governed by our novel candidate. We identified a novel compound heterozygous mutations (T>P and R>H) in a gene, belongs to a family of molecular chaperone, as LCA-causative. Two children who have both T>P and R>H mutation exhibited macular degeneration, a hallmark of LCA, indicating the foveal hypoplasia. Other family members having either one of these mutation showed normal macula. Protein structure prediction pointed out structural decay(s) in both mutants. Molecular biological analyses revealed rapid degradation of T>P mutant, while R>H mutant showed more aggregability. Protein quality control of one of the G-proteins which is governed by newly identified LCA-causative molecule was deteriorated under mutant(s) over expression. Whole-exome analysis identified a novel gene responsible for LCA. Dysfunction of this newly identified mutation affects the dynamics of one of the G-proteins which is abundant in photoreceptor, a probable trigger of LCA in this family.

IRB Status: Approved

Disclosures:

YURIKO MINEGISHI: No financial relationships to disclose

TIME COURSE OF ACTIVATION AND RECOVERY FROM ADAPTATION TO A BACKGROUND LIGHT IN MOUSE RODS

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By altering the cascade of phototransduction in steady light, photoreceptors decrease their sensitivity and alter the time course of their responses in a process known as background adaptation. We investigated the time course of onset and recovery of adaptation to dim background light with suction-electrode recording from mouse rods. We first presented background light at several different intensities in wild-type (WT) rods and measured incremental flash responses after 100, 250, 500, and 1000 ms in successive trials. Similar measurements were done with GCAPs^{-/-}. We found that in WT sensitivity and response waveform stabilized after no more than 250 ms (faster in brighter light), but in GCAPs^{-/-} steady state occurred somewhat more slowly. For recovery, we exposed the rod to the same backgrounds for 2 min and then gave flashes at 3, 6, 9, 12, 15, 30, 60 and 120 s. For WT, sensitivity and circulating current recovered within 3-6 s, but response waveform recovered slower. This difference in the time course of recovery

for sensitivity and wave form is similar to that reported by Krispel et al (JGP 122:703-712, 2003), but we used much dimmer adapting light. In GCAPs^{-/-}, sensitivity recovered more slowly than WT even after very dim light exposure; waveform recovered even more slowly than sensitivity. We conclude that the onset of adaptation is rapid even in rods lacking cyclase modulation, indicating the necessity of a second messenger of background adaptation in mammalian rods. The time courses of onset and recovery of sensitivity and response waveform are slower in GCAPs^{-/-} than in WT rods, indicating a role of the cyclase in the kinetics of adaptation. The consistent difference in the time course of recovery of sensitivity and waveform suggests that these two phenomena may be controlled at least in part by separate mechanisms.

IRB Status: None

Disclosures:

ALA MORSHEDIAN: No financial relationships to disclose

P222

WNT/B-CATENIN SIGNALING IN MICROVASCULAR ENDOTHELIAL AND MÜLLER CELLS IS ESSENTIAL FOR RETINAL VASCULAR DEVELOPMENT AND REPAIR

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Activation of Wnt/b-catenin signaling by several factors is essential for normal retinal vascular development. Here we wondered which role microvascular endothelial and Müller cell-derived Wnt/b-catenin signaling has on the development of the retinal vasculature and its changes during pathological vascular processes. Mice with an inducible conditional b-catenin deficiency in microvascular endothelial (Cdh5CreERT/Ctnnb1/fl) and Müller cells (Slc1a3CreERT/Ctnnb1/fl) were generated. To analyze vascular changes, mice were perfused with FITC-coupled dextran and retinal whole mounts were isolated. Further on, an oxygen-induced retinopathy (OIR), the model of retinopathy of prematurity in mice, was induced. In addition, mRNA expression of IGF-1 and angiopoietin-2 in retinæ from Slc1a3CreERT/Ctnnb1/fl mice was investigated. During development of Cdh5CreERT/Ctnnb1/fl mice, a retarded development of the superficial and deep vascular plexus was observed compared to control littermates. In contrast, in Slc1a3CreERT/Ctnnb1/fl mice, only slight changes in the developing retinal vasculature, but a delayed regression of the hyaloid vasculature were detected. Following an OIR in mice with a b-catenin deficiency in microvascular endothelial or Müller cells, a reduced vessel regrowth into vaso-obiterated areas and a retarded development of intraretinal vessels were detected at postnatal day (P) 17. Further on, in Slc1a3CreERT/Ctnnb1/fl mice the formation of preretinal tufts was increased by 2.6-fold compared to control littermates. In contrast, in Cdh5CreERT/Ctnnb1/fl mice the number of tufts was reduced by 25% when compared to control animals. Further on, following an OIR at P15, in retinæ from Slc1a3CreERT/Ctnnb1/fl mice mRNA levels for IGF-1 and angiopoietin-2 were significantly reduced compared to littermate controls. Wnt/b-catenin signaling in

microvascular endothelial and Müller cells is essential for the development of the retinal vasculature and for vascular repair following OIR. In mice with b-catenin deficiency in Müller cells, the effects are most likely mediated via a decreased expression of angiogenic factors such as IGF-1 and angiopoietin-2.

IRB Status: None

Disclosures:

BIRGIT MÜLLER: No financial relationships to disclose

P223

EXOGENOUS α B-CRYSTALLIN PROMOTES TUBULOGENESIS IN HUMAN RETINAL ENDOTHELIAL CELLS

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Endogenous α B-crystallin has been shown to be an important regulator of angiogenesis. Here we show that exogenous α B-crystallin plays a role in tubulogenesis of human retinal capillary endothelial cells (HREC). HREC were isolated from 45-55 year old non-diabetic donor eyes and cultured. Cells were seeded on plates coated with a basement membrane extract that had low levels of growth factors. Cells were cultured for 16 to 24 hrs with either serum-free medium or serum-free medium supplemented with endothelial cell growth factors. Tubulogenesis was assessed by staining endothelial cells with Calcein AM followed by measuring total tube length. HREC cultured with growth factors showed significantly higher levels of tubulogenesis compared to those cultured without the growth factors. α B-Crystallin depleted medium (in the presence of growth factors) showed significantly lower levels of tubulogenesis. A concentration-dependent increase in tubulogenesis was observed in cells treated with exogenous recombinant α B-crystallin, and such an effect was absent in chaperone-compromised R120G mutant α B-crystallin and ovalbumin. HREC overexpressing α B-crystallin displayed higher and HREC treated with an siRNA for α B-crystallin showed lower levels of tubulogenesis relative to control cells. The negative effect of siRNA was partially reversed by the addition of α B-crystallin. Externally added α B-crystallin increased the expression of α B-crystallin, NF- κ B nuclear translocation, cell proliferation and Akt phosphorylation in HREC. Cells treated with either VEGF-A or FGF-2 along with α B-crystallin showed higher levels of tubulogenesis than cells treated without α B-crystallin. The chaperone peptide of α B-crystallin mirrored the effects of the whole protein, whereas the scrambled peptide did not. We also found α B-crystallin to be highly upregulated in diabetic mouse retina. Taken together, our study suggests that α B-crystallin could promote retinal angiogenesis during proliferative diabetic retinopathy and pharmacological inhibition of α B-crystallin's function could be of therapeutic benefit in diabetic retinopathy.

IRB Status: None

Disclosures:

ROOBAN NAHOMI: No financial relationships to disclose

P224**DIFFERENTIAL EXPRESSION OF MIRNAS IN ROD PHOTORECEPTORS AND MÜLLER GLIA**

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During development of the retina, retinal progenitor cells give rise to 6 different types of neurons, and 1 glial cell. This process requires the expression of genes that confers specific functions and identity to each cell. miRNAs have the ability of modulate hundreds of gene targets at the post-transcriptional level making them important regulators of cell identity and physiology. Previous works have report the miRNAs expression profile in retina, but is still necessary to further define said profiles on individual cell populations. In this work, we suggest a subset of miRNAs that may be relevant in cell identity and function of rods and Müller cells. We isolate postmitotic CD73+ rods and Müller glia; we then analyzed their miRNA profile expression by microarrays, and confirm them by qPCR. In rods, our results are consistent with previous studies that reported expression of miR-183, -182, -96 -124a, -9* and -7a. In Müller glia, we found high expression levels of miR-29a, -214, -199b, and -143. This study represents a progress in the establishment of miRNA signature in individual retinal populations.

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IRB Status: International

Disclosures:

HEBERTO QUINTERO: No financial relationships to disclose

P225**DTGR: A NEW RAT MODEL FOR SYSTEMIC HYPERTENSIVE RETINOPATHY**NADINE REICHHART¹, Christina Herrspiegel¹, Sergej Skosyrski¹, Nadine Haase², Ralf Dechend¹, Olaf Strauss¹CHARITÉ UNIVERSITÄTSMEDIZIN BERLIN¹; MAX DELBRUECK CENTRE²

Systemic and local RAS play important roles in the development of hypertensive end-organ damage. To understand the pathomechanisms underlying hypertensive retinopathy, we used a double transgenic rat model (dTGR), which expresses both the rat and human RAS. The model leads to severe end-organ damage with animals dying by week 7 due to hypertension and overstimulation of systemic RAS. Purpose of the study is to assess whether systemic hypertension affects retinal structure and function. By using flatmount preparations, immunohistochemistry of paraffine sections and PatternERG we analysed the retinal phenotype of dTGR. Flatmount preparations of the retina revealed focal avascular lesions as well as pathological vessels with irregularities in shape and diameter compared to control animals. In sagittal sections of the retina we detected a focal loss of retinal ganglion cells. GFAP staining revealed activation of astrocytes in the ganglion cell layer (GCL) and

astrocytosis of the optic nerve. VEGF-positive staining could be detected in the ganglion cell layer (GCL) and the inner nuclear layer (INL). To further substantiate the loss of ganglion cells detected in the paraffine sections, Pattern ERG was carried out. By using different pattern sizes (15° and 5-7°) at 2.4hz of the appearing black and white checkerboard we showed that the positive component after about 50ms (P50) of the Pattern ERG was significantly smaller in dTGR compared to control rats at 5-7° of the displayed pattern whereas the P50 at 15° remained unchanged. This indicates a loss of ganglion cells in the retina. Thus we present a first animal model for hypertensive retinopathy showing functional and structural alterations of the retina. Astrocytosis and VEGF-A production in the inner retina indicate possible pathomechanisms for further investigation.

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Disclosures:

NADINE REICHHART: No financial relationships to disclose

P226**MICRO RNAS ASSOCIATED WITH A MODEL OF RETINAL DEGENERATION**KARTIK SAXENA¹, Riccardo Natoli², Matt Rutar³, Jan Provis²AUSTRALIAN NATIONAL UNIVERSITY¹; AUSTRALIAN NATIONAL UNIVERSITY, ANU MEDICAL SCHOOL²; AUSTRALIAN NATIONAL UNIVERSITY, JOHN CURTIN SCHOOL OF MEDICAL RESEARCH³

We have previously identified by microarray analysis a number of non-coding RNAs which are modulated in a light damage (LD) model of retinal degeneration (RD) (Natoli et al 2010). In this study, we aimed to confirm those findings and identify potential miRNAs associated with the inflammatory response to LD in Sprague-dawley (SD) rat retinas. SD rats were exposed to white light (1000lux) for 24hrs. cDNA from 5 retinas was hybridised to TaqMan® microRNA array cards to quantify the expression of ~700 miRNAs. Partek Genomics Suite 6.6 was used for bioinformatics analysis. QPCR was performed using TaqMan® hydrolysis probes. Müller cells *in vitro* were exposed to 10ng/ml IL-1b for 12hrs and miRNA expression analysed by QPCR. Statistical analysis of the cumulative expression data shows a clear difference in the miRNA expression profile between LD and control groups. We identified 37 miRNAs (p<0.05, Fold Change >2) that are differentially regulated in the LD model, including 15 miRNAs not previously identified in the retina. A majority of miRNAs, including some implicated in inflammation, were upregulated in our model with peak expression occurring in the post light exposure period. Retinal pigment epithelium (RPE) and Müller cells treated with the inflammatory cytokine IL-1beta *in vitro* showed upregulation of one of the miRNAs, miR-155. The results indicate that miRNAs, in particular miRNA-155, are modulated by LD and may be a component of the inflammatory response. Identification of the mechanisms by which these miRNAs exert their effect in the retina may lead to development of novel miRNA-based therapy for RD.

Riccardo Natoli et al, 2010, *Molecular Vision*;16:1801-1822

IRB Status: Approved

Disclosures:

KARTIK SAXENA: No financial relationships to disclose

P227

IMMUNOPROTEASOME ROLE IN STRESS RESPONSE AND TEMPORAL PTEN/AKT SIGNALING AFTER OPTIC NERVE CRUSH (ONC)

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The Immunoproteasome is highly expressed in cells of the immune system, but expressed at low basal levels in cells outside this system. Disease, oxidative stress, and inflammatory cytokines have all been shown to significantly up-regulate Immunoproteasome expression, which suggests an alternative role for the Immunoproteasome in stress signaling rather than its canonical role in assisting with MHC I antigen presentation. The signaling pathways that are affected by the up-regulation of the Immunoproteasome remain elusive, with conflicting evidence suggesting a role for the Immunoproteasome in NF- κ B signaling, one of the main stress response pathways. One well known pathway upstream of NF- κ B is the PI3K/AKT signaling axis, which is responsible for mediating cellular survival and is modulated after optic nerve crush (ONC). The purpose of this study is to determine the role of the Immunoproteasome in the retina after injury with a focus on the PI3K/AKT signaling pathway. To test the hypothesis that the Immunoproteasome assists in regulating PI3K/AKT signaling, we used WT and KO mice lacking either one (*Imp2*^{-/-}) or two (*Imp7*^{-/-}/*mecl*^{-/-}) catalytic subunits of the Immunoproteasome. Furthermore, an analysis of PI3K/AKT signaling after insulin growth factor (IGF) treatment of retinal pigment epithelial (RPE) cells derived from WT and Immunoproteasome KO mice was conducted. Our results show that mRNA and protein levels of the Immunoproteasome subunits are significantly up regulated in WT retinas following ONC. Mice lacking the Immunoproteasome subunits show either a delayed and dampened apoptotic response compared to WT mice after ONC as well as altered AKT signaling. This study links the inducible expression of the Immunoproteasome following retinal stress to pro-survival pathways, which is important in certain disease pathways.

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Disclosures:

NATHAN SCHULD: No financial relationships to disclose

P228

REPAIR OF RHODOPSIN MRNA BY SPLICEOSOME-MEDIATED RNA TRANS-SPLICING: A NEW APPROACH FOR AUTOSOMAL DOMINANT RETINITIS PIGMENTOSA

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Retinitis pigmentosa (RP) is a group of hereditary retinal dystrophies leading to blindness, due to the degeneration of photoreceptor cells and for which there is currently no treatment. The most frequent cause of RP is the occurrence of point mutations in the Rhodopsin (RHO) gene. In this context, the mutant protein causes a gain of function or dominant negative effect, deleterious for photoreceptors. Variations in the RHO expression level could also be deleterious for the retina. Therapeutic strategy should thus both lead to suppression of mutant protein expression and restoration of the normal one, at a physiological level. Spliceosome-mediated RNA *trans*-splicing allow respecting these constraints by repairing RHO mutations at post-transcriptional level. This approach consist in introducing by gene transfer an exogenous RNA — called PTM, for Pre-*Trans*-splicing Molecule — able to bind the pre-mRNA and promote the splicing in *trans*, leading to the replacement of the mutated part of the RHO pre-mRNA. We engineered 14 different RHO-PTMs able to repair any mutation in RHO exons 2, 3, 4 and 5, which differ only on the binding sequence to the endogenous RNA. To determine the efficiency of each PTM, we transiently co-transfected HEK293T cells with a plasmid encoding a PTM and another encoding the wild-type (WT) or mutant RHO. The maximum *trans*-splicing efficiency observed at the mRNA level was about 25%. We improved this efficiency to 40% by adding an intron into the cDNA replacement sequence of this PTM. We then tested PTM on HEK293T cell lines stably expressing WT or mutated RHO, created by lentiviral transduction. While WT RHO was localized to the plasma membrane, mutated RHO was retained inside the cell. We thus were able to quantify *trans*-splicing rate at the protein level by demonstrating the re-localization to the plasma membrane of repaired RHO.

IRB Status: None

Disclosures:

ADELINE BERGER: No financial relationships to disclose

P229

DIFFERENTIATION OF 3D RETINA-LIKE STRUCTURES FROM PLURIPOTENT STEM CELLS

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The majority of diseases resulting in visual impairment do so as a consequence of a loss of function of the neural retina at the back of the eye. The vast majority of patients experiencing visual disease are suffering from degenerative diseases, like age-related macular degeneration (AMD), and hereditary diseases, like retinitis pigmentosa (RP). At present, our understanding of the mechanisms involved with the progression of such diseases is still poorly understood. It is hoped that production of retinal structures using pluripotent stem cell technology, *in vitro*, will yield better models from which to elucidate the mechanisms of retinal disease, and in doing so produce novel treatments. This study therefore aimed to produce retinal structures via a non-adherent, 3-dimensional, stem cell culture system, to drive retinal differentiation. A number of different conditions were investigated, including: variation of the size of the 3D embryoid bodies initially produced to induce differentiation from pluripotent cells, addition of various signaling agonists/antagonists thought to encourage development of the retinal lineage (Wnt/Insulin pathways), variation of the serum content in culture, as well as the concentration of Matrigel™ used to coat the 3D structures. The best method employed a three step protocol blocking wnt signaling, followed by hedgehog pathway agonism and finally retinoic acid treatment. Embryoid bodies consisting of 9000 cells were observed to develop into structures best resembling native retina, characterized by the expression of known retinal markers, including PAX6, RAX, OTX2 and CHX10. Higher concentrations of Matrigel™ (2%) were found to support more robust retinal differentiation. Perhaps unsurprisingly, the capacity for retinal differentiation varied greatly depending upon the particular batch of both serum and Matrigel™ used. This study gives encouraging evidence that it may be possible to produce intact retinal structures for modeling of retinal disease.

IRB Status: None

Disclosures:

MATTHEW SMART: No financial relationships to disclose

P230

RDS GLYCOSYLATION; A WINDOW INTO THE DIFFERENTIAL ROLE OF RDS IN RODS AND CONES

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The photoreceptor specific glycoprotein retinal degeneration slow (RDS, also called PRPH2) is necessary for the proper formation of both rod and cone photoreceptor outer segments. Mutations in RDS cause both rod and cone dominant retinal disease, and it is well established that both cell types have different requirements for RDS. However, the molecular mechanisms for this difference remain unclear. Although RDS glycosylation is highly conserved, previous study revealed no apparent function for the glycan in rods. In light of the highly conserved nature of RDS glycosylation we hypothesized that it is important for RDS function specifically in cones, and could underlie part of the differential requirement for it in the two photoreceptor subtypes. Therefore we generated a knock-in mouse expressing RDS which lacks the single N-glycosylation

site (N229S). Native regulation of the mutant allele and absence of glycosylation were confirmed using northern blot/qRT-PCR and western blot. N229S animals exhibited normal rod outer segment structure and function and normal levels of RDS and the non-glycosylated RDS binding partner rod outer segment membrane protein-1 (ROM-1). However cone electroretinogram responses were decreased by 40% at 6 months of age. Since cones make up only 3-5% of photoreceptors in the WT background, N229S mice were crossed into the *nrl*^{-/-} background (in which all rods are converted to cone-like cells) for biochemical analysis. In N229S/*nrl*^{-/-} retinas, RDS and ROM-1 levels were decreased by 63% and 70%, respectively. These data suggest that the glycosylation of RDS is specifically required for RDS function or stability in cones. This difference may be due to the localization of the glycan: N229 resides in the extracellular space in cones and in the intradiscal space in rods. Further exploration of the role of RDS glycosylation may facilitate our understanding of the differential function of RDS in rods versus cones.

IRB Status: None

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MICHAEL STUCK: No financial relationships to disclose

P231

THE ROLE OF GAPDH/SIAH1 IN HUMAN RETINAL PERICYTE APOPTOSIS

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Diabetic Retinopathy (DR) is the leading cause of blindness in working age Americans. Current therapies for DR address only the latest stages of the disease, are invasive and are of limited effectiveness. Retinal pericyte death is one of the earliest pathologic features of DR. Though early pericyte apoptosis has been observed in diabetic patients and in animal models of DR, the cause of pericyte death remains unknown. A novel pro-apoptotic pathway initiated by the interaction between GAPDH, and the E3 ubiquitin ligase, seven in absentia homolog 1 (Siah1), was recently identified. The goal of the present study was to determine the role of the Siah1/GAPDH complex in pericyte apoptosis. Annexin V and Caspase-3 activity and expression levels was used to quantify apoptosis levels in human retinal pericytes (HRP) exposed to DR relevant stimuli. HRP were also treated with R-(-) Deprenyl (100nM) or Siah1 siRNA (10µM) in the presence of absence of D-glucose (30mM). For Western blot analysis, HRP were lysed and nuclear and cytoplasmic proteins extracted. Western blots were quantified using Image J. Siah1 and caspase-3 mRNA levels were assayed by qRT-PCR. FACS analysis revealed that LPS, TNFα and high glucose cause HRP apoptosis. Culture media supplemented with high glucose or TNFα led to significant upregulation of Siah1 total protein levels. R-(-)-Deprenyl and Siah1 siRNA inhibited this high glucose-induced Siah1 upregulation. In addition, high glucose resulted in GAPDH nuclear translocation and was inhibited by R-(-)-Deprenyl. High glucose and TNFα caused an increase in caspase-3 enzymatic activity, and this was inhibited by R-(-)-Deprenyl. Lastly, high glucose resulted

in an increase of caspase-3 expression, and this was inhibited by Siah1 siRNA. Our findings suggest that GAPDH/Siah1 may link HRP apoptosis to diabetes-relevant stimuli. Future experiments will examine the role of GAPDH-nuclear translocation and HRP death in an animal model of DR.

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P232

NEURONAL VHL DELETION INDUCES PERSISTENT FETAL VASCULATURE AND STRONG SUPPRESSION OF RETINAL VASCULAR FORMATION

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Purpose: von Hippel-Lindau protein (VHL) and its target hypoxia-inducible factors (HIFs) play important roles in the development, physiology, and pathophysiology of many tissues. In general, HIF stabilization and activation (caused by VHL deletion) induces pro-angiogenic gene expression. Previously, we have reported activities of VHL and HIF in neurons, astrocyte, myeloid cells, and pigment epithelium cells during development and maturation of the retina (*J Cell Biol.* 2011, *J Clin Invest.* 2012). However, the role of VHL in vascular development is still not well understood. In this study, retinal vascular development regulated by neuronal VHL was investigated utilizing retinal pan-neuronal conditional knockout mice.

Method: CRX-Cre mice were crossed with ROSA26-GFP reporter mice to confirm the expression of cre recombinase. Retinal neuronal specific conditional VHL knockout mice were obtained by crossing CRX-Cre mice with VHL^{flxed}/flox mice (VHL;CRX-Cre mice). Fundus photography, immunohistochemistry, and histology were used to examine the phenotype of these mice.

Results: CRX-Cre;ROSA26 mice expressed GFP in all neurons throughout the entire retina. Surprisingly, VHL;CRX-Cre mice revealed an arrest of retinal vascular development despite strong HIF stabilization in these cells. On the other hand, VHL;CRX-Cre mice showed proliferative hyaloidal vascular endothelial cells on the surface of the retina as well as photoreceptor degeneration. These phenotypes resemble persistent fetal vasculature (PFV), resembling that observed in developmental human retinal disease.

Conclusion: These data suggest that neuronal VHL is critical for proper vascular and neuronal development in the retina. The VHL-HIF cascade could be targeted to prevent or treat retinal developmental anomalies such as PFV.

IRB Status: Approved

Disclosures:

YOSHIHIKO USUI: No financial relationships to disclose

P233

PHOTORECEPTOR REGENERATION IN TRANSGENIC MICE
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Photoreceptor regeneration in the mammalian eye remains an elusive goal. Our laboratory is investigating the possibility of tweaking the RPE, a tissue with naturally occurring wound healing ability, for photoreceptor regeneration in situ in the eye. The underlying theme is to use a regulatory gene with pro-photoreceptor activity to reprogram RPE cells to initiate photoreceptor differentiation, so as to channel RPE's well-known capacities of proliferation and plasticity towards photoreceptor production inside the mammalian eye. Previous study showed that transgenic mice (generated with DNA that would express *ngn1* or *ngn3* in RPE cells driven by PRPE65 or PVMD2) contained photoreceptor-like cells in the subretinal space. In this study, we examined whether new photoreceptor cells could be born in adult transgenic mice. We performed daily intraperitoneal injection of BrdU for 2 weeks into mice transgenic (Tg) for the expression of transcription factor neurogenin1 (or 3) driven by RPE promoter (PRPE65 or PVDM2) and Tg/*rd1* mice (generated by crossing the Tg mice with *rd1/rd1* mice). Double-immunohistochemistry for BrdU incorporation and photoreceptor protein recoverin detected BrdU+/recoverin+ cells in the Tg mice aging between 7 weeks and 1 year. Most of the double-labeled cells localized within the outer nuclear layer of the periphery retina. Occasionally, double-labeled cells were found to be attached to the RPE or containing dark pigment granules typically present in RPE cells, reminiscent of being in a RPE-to-photoreceptor transitional stage. BrdU+/recoverin+ cells were also detected in 7 week-old and 5 month-old Tg/*rd1* mice. Our data suggests that new photoreceptor cells could be produced in adult transgenic mice, conventional and Tg/*rd1*.

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Disclosures:

SHU-ZHEN WANG: No financial relationships to disclose

P234

COMPLEMENT DEPOSITION IN THE RETINA OF RPE-SPECIFIC CFH-/- AND CFH-/-CFB-/- DOUBLE KNOCK-OUT MICE

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Dysregulation of the complement system plays an important role in the development of age-related macular degeneration (AMD). Previous studies in knockout mice have investigated the effects of deletion of CFH or CFB on the retina, but here we used *Cfh*-/*Cfb*-/ double knock-out (DKO) mice to study the

functional interplay between CFH and CFB. Additionally we created mice where *Cfh* was deleted in the retinal pigment epithelium (RPE) to gain insight into the role of local CFH production in the retina. Wild type (WT) retinal sections stained for C3 and its breakdown products C3b, iC3b and C3c revealed C3 in retinal vessels and along the basal surface of the RPE where C3 breakdown products were also detected. Loss of CFH in *Cfh*^{-/-} mice caused secondary depletion of C3 from the serum, and in the retina staining of C3 and its breakdown products was absent along the basal surface of the RPE. However, in RPE-specific *Cfh*^{-/-} mice, circulating C3 returned to normal levels and could now be identified in retinal vessels and along the basal surface of the RPE as in WT mice. Deletion of both *Cfb* and *Cfh* in *Cfh*^{-/-}*Cfb*^{-/-} DKO mice also restored circulating C3 levels, however this reversal of phenotype was not observed in *Cfh*^{-/-}*Cfb*^{+/-} mice where the phenotype observed was similar to *Cfh*^{-/-} mice. *Cfh*^{-/-}*Cfb*^{-/-} mice differed from WT mice in having reduced staining for C3 breakdown products at the basal surface of the RPE. Overall our results show that accumulation of C3 along the basal surface of the RPE is dependent on the presence of C3 in the serum, which is depleted in *Cfh*^{-/-} mice but restored in RPE-specific *Cfh*^{-/-} mice. The accumulation of C3 breakdown products is largely dependent on activation of the alternative pathway since it is reduced in the absence of CFB.

IRB Status: None

Disclosures:

JENNIFER WILLIAMS: No financial relationships to disclose

P235

REVERSAL OF OXIDATIVE STRESS IN A MOUSE MODEL OF DRY AMD

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Retinal cell death due to oxidative stress is a contributing factor for development of age related macular degeneration (AMD) which causes vision loss among millions of people in industrialized nations. We established a model of RPE (retinal pigment epithelium) oxidative stress by Cre-lox mediated deletion of the Sod2 gene, that codes for the protective enzyme manganese superoxide dismutase (MnSOD), leading to some of the features of geographic atrophy. Our aim is to determine whether delivery of Sod2 using adeno-associated virus (AAV) can prevent retinal degeneration seen in these mice and whether gene therapy can prevent degeneration once it has begun. Deletion of Sod2 was induced by doxycycline treatment of mice with a "floxed" allele of Sod2 and an RPE-specific tet-transactivator controlling expression of Cre. Retinal degeneration was monitored by electroretinography (ERG) and spectral domain optical coherence tomography over a period of 9 months. Mouse Sod2 with a Myc epitope under the control of a small chicken beta-actin promoter (smCBA) was packaged into self-complementary AAV vector and injected

into right eye. Deletion of Sod2 in the RPE leads to some of the salient features of dry AMD as they showed a gradual decline in the ERG response and thinning of the outer nuclear layer (by SD-OCT) which were statistically significant by 6 months. MnSOD expression was detected in RPE and negligible expression was seen in the neural retina. We are testing whether this Sod2 vector can be used as a tool to reverse oxidative stress in the mouse model of dry AMD.

IRB Status: None

Disclosures:

MANAS BISWAL: No financial relationships to disclose

P236

THE ZEBRAFISH AS A MODEL SYSTEM FOR THE ANALYSIS OF MELANOSOME BIOGENESIS IN THE RPE IN HEALTH AND HUMAN DISEASE

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Melanosome biogenesis in mammalian RPE is difficult to study because of the short time window for melanosome biogenesis in embryonic life and the scarcity of RPE cell culture systems that make melanosomes. Therefore, we have investigated the regulation of melanosome biogenesis in the RPE of the zebrafish because it is a genetically tractable model allowing the depletion of selected proteins using morpholinos at a time when melanosome biogenesis is highly active in the RPE. The aim of this project was to investigate melanosome maturation and the potential to utilize zebrafish as a model to study human disease affecting RPE melanosomes. Immature melanosomes were found to be difficult to identify using electron microscopy in zebrafish RPE, despite the intense melanosome production from 2 to 5 days post fertilization. Depleting tyrosinase to inhibit melanin synthesis revealed the presence of immature melanosomes containing striations on which traces of melanin had been deposited. This suggests melanosomes in zebrafish RPE mature in a similar manner to those in mammalian RPE, leading to the formation of elongated melanosomes that can access the RPE apical processes. By depleting both tyrosinase and PMEL (a protein required for striation formation in mammalian melanosomes), we were able to examine the relationship between melanosome size, shape and movement in the RPE. The similarities between zebrafish and mammalian melanosome biogenesis suggests that the zebrafish could be a useful model for human melanosome biogenesis disorders. Therefore, zebrafish were depleted of OA1, mutations in which are the most common cause of ocular albinism in human patients. The OA1 depletion was found to cause a reduction in melanosome number before any effect of melanosome size, phenocopying the OA1 knockout mouse. This result emphasizes the potential to study human RPE melanosome maturation defects in zebrafish.

IRB Status: None

Disclosures:

THOMAS BURGOYNE: No financial relationships to disclose

CIGARETTE SMOKE AND NRF2 DEFICIENCY IMPAIRS MITOCHONDRIAL FUNCTION IN RETINAL PIGMENT EPITHELIAL (RPE) CELLS

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Cigarette smoking (CS) is the strongest epidemiologic risk factor for AMD. Nrf2, a transcriptional factor that controls a cascade of antioxidant genes, decreases with age and prolonged CS. Mitochondria are a major source of ROS, which can be increased by CS. The study's purpose was to determine the effect of CS and Nrf2 on mitochondria and cell function. ARPE-19 cells were treated with 100-500ug/ml CS extract (CSE) and Nrf2 knock-down (KD). While cells remained viable, CSE induced protein carbonylation and a cytoprotective unfolded protein response (UPR). Mitochondrially produced ATP decreased while superoxide increased in a dose-dependent manner. CSE increased the expression of TRX2, PRX3, and SOD2 after treatment with 100ug/ml CSE, but were not further increased at higher doses while SRX increased in a dose-dependent fashion. We conclude that high CSE induces oxidative damage and mitochondrial dysfunction, but not to the extent that it affects viability. We next knocked down Nrf2 and observed a dose dependent decrease in viability of up to 35% at 500ug/ml CSE. Nrf2 KD elicited a UPR, but the addition of CSE did not further alter the UPR. Superoxide production was magnified with Nrf2 KD, but the addition of CSE did not further increase the generation of mitochondrial superoxide. Nrf2 KD magnified the decrease in ATP induced by CSE, but had no impact on the expression of TRX2, PRX3, and SOD2 while the expression of SRX was decreased at all CSE doses. Nrf2 KD with CSE promoted cytochrome c release. In conclusion, RPE cells exposed to CSE generated a protective antioxidant and unfolded protein response while mitochondria became dysfunctional in part, due to an incomplete mitochondrial specific antioxidant response. Decreased Nrf2 signaling from CSE contributed to reduced antioxidant response in both the cytoplasm and mitochondria, leading to mitochondrial dysfunction that can impair cell viability.

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Disclosures:

MARISOL CANO: No financial relationships to disclose

GENERATION OF IPS-RPE FROM PATIENTS WITH AUTOSOMAL DOMINANT VITREORETINOCHOROIDOPATHY

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Bestrophinopathies are a group of clinically distinct inherited diseases that lead to the gradual loss of vision in and around the macular area. There are no treatments for patients suffer-

ing from Bestrophinopathies and no measures can be taken to prevent visual deterioration in those who have inherited disease-causing mutations. These diseases, caused by mutations in the retinal pigment epithelium (RPE) specific gene Bestrophin 1 (BEST1), affect the function of the RPE leading to the death of overlying retinal cells and subsequent vision loss. We have previously shown that RPE can be readily differentiated from induced pluripotent stem cells (iPSCs), making them a powerful system in which to investigate inherited diseases of the RPE, such as bestrophinopathies. In this study we have recruited patients with autosomal dominant vitreoretinopathy (ADVIRC) who have been accurately phenotyped and express a missense BEST1 substitution (pVal235Ala), thought to result in exon skipping. We have cultured fibroblast cells from donated skin biopsies and reprogrammed the cells into iPSCs using episomal vectors (Oct4, Sox2, Klf4, Lin28, c-myc). Pluripotency was confirmed using immunostaining of iPSC colonies and trilineage verified by PCR assays following embryoid body differentiation. iPSCs were differentiated into RPE cells using our spontaneous differentiation method, purified by manual dissection and seeded as single cells to form an RPE monolayer. The purified RPE were assessed by standard protocols including immunocytochemistry, western blot and PCR. We have shown that skin biopsies from ADVIRC patients can be used to create RPE using iPSC technology. These cells will be used in future studies as disease models and as a platform for drug discovery to treat bestrophinopathies.

IRB Status: International

Disclosures:

AMANDA-JAYNE CARR: No financial relationships to disclose

HUMAN ORGANIC ANION TRANSPORTING POLYPEPTIDE 1A2 (OATP1A2) IS A NOVEL MEDIATOR OF CELLULAR UPTAKE OF ALL-TRANS-RETINOL IN HUMAN RPE CELLS

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Vision in all vertebrates depends on an exchange of retinoids between the retinal pigment epithelium (RPE) and photoreceptors. Defects in any steps of the retinoid cycle can lead to retinal degeneration. All-trans-retinol (atROL) plays an important role in visual signal transduction within the visual cycle. However, how atROL enters human RPE cells from the interphotoreceptor matrix remains unclear. This study investigated the role of human organic anion transporting polypeptide 1A2 (OATP1A2) in the uptake of atROL in human RPE cells. Immunoblotting and immunofluorescent staining were conducted to evaluate the expression and localization of this transporter in human RPE cells. Transporter function and inhibition studies were conducted to assess the interaction of

this transporter with atROL. Our study revealed, for the first time, that OATP1A2 is expressed in human RPE cells, mainly localized to the apical membrane of the RPE monolayer. Our data also indicated that atROL can potentially inhibit the uptake of the classical OATP1A2 substrate, estrone-3-sulfate (ES3), in over-expressing cells with the IC50 value of $3.6 \pm 0.9 \mu\text{M}$. Our uptake with 3H-atROL in over-expressing cells revealed that atROL is a novel substrate of OATP1A2. We then confirmed these findings in primary human RPE cells, which showed that ES3 and atROL could mutually inhibit the uptake of each other. Furthermore, the transport of both substrates was diminished in primary RPE cells with OATP1A2 knockdown. Taken together, our study provided the first evidence of OATP1A2 expression in human RPE cells. More importantly, we reported the novel role of OATP1A2 in cellular uptake of atROL in RPE cells, which is essential to retinal retinoid exchange. Our study contributes to a greater understanding of the underlying molecular mechanisms of retinoid transport between RPE cells and photoreceptors and novel insights into potential pharmaceutical interventions for retinal degenerations associated with visual cycle disruption.

IRB Status: None

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P240

MAJOR AMERICAN DIETARY PATTERNS ARE RELATED TO RISK OF AGE-RELATED MACULAR DEGENERATION

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We hypothesized that major American dietary patterns and differences in specific nutrient intakes, including vitamins C and E, beta-carotene, zinc, lutein/zeaxanthin, docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA), within the context of the patterns are associated with age-related macular degeneration (AMD) risk. 8,103 eyes from 4,088 eligible participants in the baseline Age-Related Eye Disease Study (AREDS) were classified into control (n=2,739), early AMD (n=4,599), and advanced AMD (n=765) according to the AREDS AMD Classification System. Using food consumption data collected by a 90-item food frequency questionnaire, two major dietary patterns, prudent and Western patterns, were identified by factor analysis based on 37 food groups. The "prudent pattern" was characterized by higher intake of vegetables, legumes, fruit, whole grains. The "Western pattern" was characterized by higher intake of red meat, processed meat, high-fat dairy products, French fries, refined grains. For early AMD, the multivariate-adjusted odds ratio (OR) comparing the highest to lowest quintile of the prudent pattern score was ORE5P=0.74 (95% confidence interval (CI): 0.59–0.91; P_{trend}=0.01), and the OR comparing the highest to lowest quintile of the Western pattern score was ORE5W=1.56 (95% CI: 1.18–2.06; P_{trend}=0.01). For advanced

AMD, the ORE5P was 0.38 (95% CI: 0.27–0.54; P_{trend}<0.0001), and the ORE5W was 3.70 (95% CI: 2.31–5.92; P_{trend}<0.0001). Our data also suggested benefit from higher dietary intakes of vitamin C, lutein/zeaxanthin, and beta-carotene, especially in the context of the Western pattern. In the context of the two major patterns, we did not observe a relationship between intake of vitamin E, zinc, DHA or EPA and AMD risk. This cross-sectional study indicates that diet plays an important role in the development of AMD and that the risk for AMD can be diminished by eating a prudent diet.

IRB Status: Verified

Disclosures:

CHUNG-JUNG CHIU: No financial relationships to disclose

P241

A QUANTITATIVE MODEL OF CHOROIDAL NEOVASCULARIZATION IN THE RABBIT

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Better preclinical models of choroidal neovascularization (CNV) are needed to effectively evaluate pathophysiology and new therapies. Current models are not relevant pharmacokinetically (rodent laser models), pathophysiologically (i.e., VEGF rabbit challenge models), or economically (primate laser models). The purpose of this study was to characterize, quantitatively, a laser induced CNV model in the New Zealand Black (NZB) rabbit. To determine the most effective laser power, lesions were created using 1000, 850, and 500 mw/1sec power from an 810 nm diode laser. Laser lesions were also made followed immediately by treatment with 2 mg (50 μl) intravitreal injection (IVT) of TRISENCE®. Color fundus photography, and retinal optical coherence tomography (OCT), including retinal thickness measurements, were performed on days 7 and 21. Following euthanasia, eye cups were labeled with 4',6-diamidino-2-phenylindole (nucleus), isolectin IB4 (blood vessels and microglia) and phalloidin (F-actin). The sclera-choroid/RPE complex was flat mounted, 2D fluorescent images were acquired, and the isolectin IB4 signal was used to quantify neovascularization. White retinal lesions were induced with all laser powers and remained visible for the entire study. CNV was also induced with all laser powers; however, more consistent neovascularization was observed in the flat mounts with the 500 mw (day 7) and 850 mw (day 21) laser powers. TRISENCE® reduced significantly both the retinal thickness (OCT) surrounding laser lesions and the isolectin IB4 signal 42% (p<0.007) on day 21 compared to untreated, lasered eyes. The results of this study demonstrate a quantitative and qualitative method for evaluating the pathophysiology of new therapies in the NZB rabbit. The results of this study warrant further validation for quantitative assessment of specific CNV therapies.

IRB Status: None

Disclosures:

WILLIAM CULP: Consultant/Advisor relationship with Powered Research, LLC; Employee relationship with Affinergy, LLC

CHARACTERIZATION OF BEST1 ISOFORMS AND MIRNA LEVELS BETWEEN THE HUMAN MACULAR AND EXTRAMACULAR RETINAL PIGMENT EPITHELIUM

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Best disease is an early onset macular dystrophy caused by mutations in the BEST1 gene. The molecular basis of the regional pathology of Best disease is poorly understood. The goals of this study were to determine the regional distribution of RNA transcript isoforms of BEST1, to assess DNA methylation of BEST1 CpG islands (CGI), and to evaluate expression of four miRNAs with potential to regulate BEST1 between human macular and extramacular retinal pigment epithelium (RPE). DNA or RNA was extracted from RPE/choroids from fixed or frozen human eye samples. Transcript isoforms were quantified by real-time quantitative PCR (qPCR) using isoform-specific primers. Potential BEST1 regulating miRNAs were identified bioinformatically, and miRNA levels were measured with qPCR. DNA methylation was evaluated using bisulfite-specific sequencing (BSP). Results were compared between macular and extramacular punches. The qPCR analysis from 3 pairs of regional RPE samples indicated that the relative abundance of three BEST1 transcripts (isoform-1, -3 and -4) was significantly lower in the macular than extramacular RPE (7-50 fold, respectively ($P < 0.05$)). In BSP analysis from 13 pairs of regional comparisons, methylation of BEST1 CGI was found at the 18th to 19th CpG site of its 3'-terminal, but the methylation pattern was not significantly different between the macular and the extramacular RPE. Three of four miRNAs evaluated as potential regulators of BEST1 (hsa-miR-107, hsa-miR-204 and hsa-miR-211) were expressed at higher levels in macular than extramacular RPE-choroid (6.9, 6.0, and 7.8 fold respectively ($P < 0.05$)). The lower levels of three BEST1 transcripts (isoform-1, 3 and 4) and higher levels of three miRNAs (hsa-miR-107, hsa-miR-204 and hsa-miR-211) were found in human macular RPE. This reciprocal relationship suggests that miRNAs may repress BEST1 expression in the macula. A better understanding of BEST1 regulation is likely to provide new insights into therapeutic interventions for Best disease.

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Disclosures:

SHEMIN ZENG: No financial relationships to disclose

A2E AND OTHER BIS-RETINOIDS: POTENTIAL INVOLVEMENT IN RPE PATHOBIOLOGY IN A RAT MODEL OF A HUMAN HEREDITARY CHOLESTEROL BIOSYNTHETIC DISORDER

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Hereditary defects in cholesterol biosynthesis comprise a family of devastating human recessive diseases. Key among these is Smith-Lemli-Opitz syndrome (SLOS), caused by mutations in DHCR7, the enzyme that catalyzes conversion of 7-dehydrocholesterol (7DHC) to cholesterol. AY9944 is a selective inhibitor of DHCR7; treatment of rats with AY9944 provides a SLOS animal model. Using this model, we previously reported that the retinal pigment epithelium (RPE) exhibits profound accumulation of phagosomes and other cytoplasmic inclusions, compared to controls (Fliesler et al., *Arch Ophthalmol.* 2004). We hypothesized that the RPE pathology might also entail elevated levels A2E and other *bis*-retinoids. AY9944-treated and age-matched (10-11 wk old) control rats were euthanized, and eyes (N=4 per group/treatment) were enucleated: one set was flash frozen in liquid nitrogen and stored at -80°C for biochemical analysis, while a companion set was formalin-fixed and stored at 4°C for histological analysis. HPLC analysis of A2E plus other *bis*-retinoids and all-*trans* retinaldehyde was performed as previously described (Sparrow et al., *Methods Molec Biol.*, 2010). Formalin-fixed, OCT-embedded eyes were cryosectioned, and frozen sections (unstained) were examined for RPE autofluorescence by confocal scanning laser fluorescence microscopy (488 nm excitation, 500-600 nm emission). Compared to controls, eyes from AY9944-treated rats exhibited the following fold-change increases ($p < 0.05$): A2E, 1.52; isoA2E, 2.20; total A2Es, 1.71; all-*trans* retinaldehyde, 1.57. RPE cells in AY9944-treated rat eyes also contained increased numbers of punctate, hyper-fluorescent inclusions, compared to age-matched controls, consistent in size and distribution with phagosomes derived from ingested rod outer segments. We conclude that RPE cells in the SLOS rat model contain elevated levels of A2E and related *bis*-retinoids, consistent with the observed increase in their phagosome content, compared to untreated controls. These changes may contribute to the retinal dysfunction and degeneration observed in the AY9944-induced SLOS rat model.

IRB Status: None

Disclosures:

STEVEN FLIESLER: No financial relationships to disclose

ASSESSMENT OF CALCIPOTRIOL, VITAMIN D3 AND CHOLESTEROL AS NOVEL REAGENTS IN THE REPIGMENTATION OF ARPE-19 CELLS

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Eumelanin is ubiquitous in intracellular RPE melanosomes providing photoprotection to basal structures, absorbing UV and stray light. RPE cells in vitro and the ARPE-19 cell line lack melanogenic capacity and do not to express essential melanogenic proteins. Primary tissue derived RPE gradually lose cellular melanosomes during proliferation and restoration or prevention of loss of such an intrinsic characteristic would provide cell cultures more comparable to RPE in situ. Skin repigmentation using Calcipotriol (CPT), Vitamin D3 (VD3) and Cholesterol (CHL) has been evaluated in melanocytes in vitro, in relation

to treatment of vitiligo in combination with UV stimulation. The aim of this study was to evaluate the ability of CPT, VD3 and CHL to induce melanogenesis and thus repigmentation of RPE cells. Sub-confluent ARPE-19 cells were treated with test media containing CPT (50, 25, 5-0.5µg/mL), VD3 (50, 20, 5-2ng/mL) and CHL (50, 20, 5-0.5µg/mL) in DMEM:F12 containing 10% FCS. Group 1 was exposed to UV light for 4 hours at 48, 72 and 96 hours post seeding then fixed and stained on day 7. Group 2 cultures were treated twice weekly with the test media and cultured for 8 weeks. Negative (media only) and positive (10% DMSO) controls were used. The 50µg/mL of CHL and VD3 proved cytotoxic. Samples treated with CPT (25, 5-0.5µg/mL) and VD3 (20, 5-2ng/mL) showed little morphological variation compared with the negative control. CHL (20 & 5µg/mL) treated cells showed a reduced growth rate and patchy epithelial-like morphology. CHL appeared to stimulate vesicle formation around the nuclei of cells. Autofluorescent vesicles were observed in CHL treated cultures but only occasionally in CPT, VD3 treated and negative control cultures with or without UV stimulation. Quantification of vesicle number and gene expression is now under way. The vesicles observed in CHL treated cultures could be due to stimulation of melanogenesis as demonstrated by the autofluorescence.

IRB Status: Verified

Disclosures:

EVA GRINDLEY: No financial relationships to disclose

P245

MICRORNA-21 REGULATES PRORENIN RECEPTOR (PRR)-INDUCED VEGF SYNTHESIS THROUGH MODULATING MEK/ERK PATHWAY IN ARPE-19 CELLS DURING HYPERGLYCEMIA

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Extracellular signal-regulated kinase (ERK)1/2 signaling has been reported to be involved in the release of vascular endothelial growth factor (VEGF), a key stimulator in the development of proliferative diabetic retinopathy (DR). MicroRNAs (miR/miRNA), which negatively regulate the translation of mRNAs, are involved in a large number of biological functions and diseases such as cancer, cardiovascular diseases, and diabetes. miR-21 has been reported to target ERK regulators: Sprouty homolog (Spry)1, Smad7, and phosphatase and tensin homolog (PTEN). In this study, we examined the underlying role of miR-21 in the regulation of PRR-mediated induction of VEGF and VEGFR2 expression via ERK signaling in hyperglycemic condition. In cultured ARPE-19 cells exposed to normal (NG, 5.5 mmol/l) or high glucose (HG, 33 mmol/l) for 48h, the down-stream PRR-mediated signaling pathway was investigated using perindopril (10 µmol/L, Sigma) to inhibit angiotensin converting enzyme (ACE) and siRNA to downregulate PRR expression. To examine the role of miR-21 in the regulation of ERK, cells cultured in NG or HG were transfected with negative control (scramble), miR-21 mimics, or antagomir-21. miRNAs/mRNAs and proteins

were measured by quantitative Reverse Transcriptase PCR (qRT-PCR) and immunoblotting, respectively. Compared to NG control, HG significantly induced the expression of PRR, NADPH oxidase (NOX), ERK, VEGF, VEGFR2, and miR-21, but significantly suppressed the expression of Spry1, Smad7, and PTEN. In contrast, silencing of PRR expression significantly abolished HG-induced Nox4, ERK and VEGF expression. Also, miR-21 mimics dramatically increased the expression of ERK, VEGF, and VEGFR2. However, this effect was antagonized by knocking down the miR-21 level by antagomir-21. Our findings, for the first time, showed that the pleiotropic action of miR-21 induced ERK/VEGF/VEGFR2 expression in hyperglycemic condition by simultaneously targeting multiple ERK regulators in ARPE-19 cells. Therefore, miR-21 may serve as a potential therapeutic target for diabetes-induced retinal pathology, including DR.

IRB Status: None

Disclosures:

RASHIDUL HAQUE: No financial relationships to disclose

P246

HISTOPATHOLOGY OF EYES FROM PATIENTS WITH AUTOSOMAL RECESSIVE RETINITIS PIGMENTOSA CAUSED BY NOVEL EYS MUTATIONS

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Retinitis pigmentosa (RP) is the most common form of inherited retinal degeneration, affecting 1 in 3,500 people. Sixty-percent of all RP cases follow an autosomal recessive (arRP) inheritance pattern. Mutations in the EYS gene account for approximately 5-16% of arRP cases. Here we report the retinal histopathological changes in the eyes of multiple donors from two families with arRP caused by mutations in the EYS gene. EYS encodes a protein whose function remains to be elucidated. However, the only characterized EYS homologue, *Drosophila*'s spacemaker or SPAM, is involved in the assembly of the light sensitive rhabdomere, the insect equivalent of vertebrate photoreceptor outer segments. DNA analysis of the donors revealed EYS mutations c.2259+1G>A and c.2620C>T (p.Q874X) in family 1 and c.4350_4356del7 (p.Ile1451Profs*3) and c.2739-?_3244+?del in family 2. Eyes were evaluated with macroscopic, scanning laser ophthalmoscopy (SLO) and optical coherence tomography (OCT) imaging. Perifovea and peripheral regions of the retina were processed for microscopy and immunocytochemistry with markers to cone-specific and rod-specific photoreceptor proteins. Imaging studies revealed the presence of bone spicule pigment in all arRP donor retinas. Histology of all three affected donor eyes revealed very thin retinas with little evidence of stratified nuclear layers in the periphery. In contrast, the macular area displayed a prominent inner nuclear layer. Immunocytochemistry analysis revealed near-total absence of rod photoreceptors in all arRP donor eyes. In addition, our results identified preservation of perifoveal cones in retinas with the midsize genomic

rearrangement (c.4350_4356del7 (p.Ile1451Profs*3) and c.2739-?_3244+?del)) as compared to retinas with the truncating mutations (c.2259+1G>A and c.2620C>T (p.Q874X)) and may indicate an ability to maintain some central vision for a longer duration. This comprehensive examination of postmortem eyes obtained from individuals in whom the disease-causing gene has been established offers a unique opportunity to study the relationship between genotype and disease pathogenesis.

IRB Status: Approved

Disclosures:

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P247

AGING ALTERS THE PROTEOLYTIC ACTIVITY OF HUMAN RETINAL PIGMENT EPITHELIUM: IMPLICATIONS FOR THE PATHOGENESIS OF AGE-RELATED MACULAR DEGENERATION

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Proteolysis is essential for retinal pigment epithelium (RPE) function in relation to maintaining the structure and function of its specialized surrounding tissues. We have shown that one of the most abundantly expressed RPE proteins, the cysteine proteinase inhibitor cystatin C (cysC), a variant of which has been associated with age-related macular degeneration (AMD), presents decreased expression with age in human RPE/choroid specimens. The aim of this study was to further characterize potential altered proteolytic homeostasis in the retina/choroid in response to a common age-related stress, the accumulation of advanced glycation end-products (AGEs). Human RPE cell lines ARPE19 and D407, and primary human fetal RPE (hfrPE) cells, were cultured for 14 days on *in vitro* Bruch's membrane mimics (placental ECM/matrigel), that, prior to cell seeding, were either untreated, or treated with glycolaldehyde to induce AGE formation. Expression/secretion of cysC, and potential targets, cathepsins (cats) B, L, and S in/from RPE was determined by Western blot and ELISA. We have confirmed that cysC, as well as some of its main functional targets, catB, catL and catS, are secreted by RPE cells. Following AGE exposure, cysC expression/secretion was decreased by up to 35% (independent T test, $p \leq 0.041$) compared to cells on untreated BrM mimics. CatB and catS expression was unaffected by AGEs, whereas catL expression was decreased by up to 40% ($p \leq 0.044$). Our findings suggest that the RPE maintain a level of control over extracellular proteolytic events, via the secretion of highly active proteases, and their inhibitors. Molecular stress associated with natural aging can alter this protease/inhibitor balance, which in turn has the potential to contribute to pathological features of AMD, such as the formation of toxic aggregates, and extracellular structural alterations.

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P248

IRON IS REQUIRED FOR MATURATION OF AMYLOID PRECURSOR PROTEIN IN RETINAL PIGMENTED EPITHELIAL CELLS

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Amyloid precursor protein (APP) gives rise to amyloid beta which is implicated as pathogenic in both Alzheimer's disease and age-related macular degeneration (AMD). Our studies demonstrate that retinal pigmented epithelial cells (RPE) contain an immature as well as a mature, glycosylated form of APP, while they secrete only the mature form. The mature form of APP is formed by glycosylation within the Golgi, with serial addition of mannose, n-acetylglucosamine (GlcNAc) and sialic acid residues. Since APP is claimed to be a ferroxidase, we investigated the effect of iron chelation on APP levels in and secretion by RPE. APP was determined by Western blot analysis. Iron chelation reduced the size of the mature intracellular form of APP, while having no effect on size of the immature form. We determined by combinatorial enzymatic digestion that mature APP treated with sialidase was reduced in size and matched the size of the new smaller APP found after iron chelation. This indicates an iron requirement for the addition of sialic acid. Unexpectedly, iron chelation blocked secretion of the mature form of APP and resulted in secretion of APP of a size similar to the immature form. From our enzymatic digestion studies, it is apparent that the presence of sialic acid protects the glycosylated form of APP from enzymatic removal of GlcNAc and mannose residues. Therefore, since iron chelation prevents the addition of sialic acid, the other sugars can be removed, resulting in the secretion of only the immature form of APP. The involvement of iron in protein glycosylation is a novel and significant finding with important ramifications for this essential cellular process.

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Disclosures:

M. CHRISTINE MCGAHAN: No financial relationships to disclose

P249

NOVEL PENTABLOCK COPOLYMER BASED NANOFORMULATIONS FOR SUSTAINED OCULAR DELIVERY OF PROTEIN THERAPEUTICS

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Various vision threatening disorders such as age-related macular degeneration (wet-AMD), diabetic retinopathy (DR) and diabetic macular edema (DME) require frequent intravitreal injections

of anti-VEGF antibodies or fragments thereof. Frequent intravitreal injections are associated with many complications such as secondary infections (endophthalmitis), retinal hemorrhage, retinal detachment and more importantly patient non-compliance. Therefore, development of sustained release formulation which can reduce the frequency of intravitreal injection is a viable option. The objective of this study is to evaluate novel penta block (PB) copolymers based formulations including thermosensitive gel, nanoparticles (NPs) and a composite formulation comprising NPs dispersed in gel after topical instillation and single intravitreal injection in rabbits. In this study, NP preparation method was successfully optimized to improve entrapment efficiency (EE) and drug loading (DL) of model macromolecules such as IgG and IgGFab. With this optimized method, a remarkably improved EE (~ 46% to 76%) and DL (~15% to 18%) have been observed. Results of in vitro studies depicted a nearly zero ordered release for significantly longer duration of time (~ 120 days) without showing any burst release effect. Moreover, in vitro biocompatibility assay exhibited negligible release of cytokines suggesting biocompatible nature of PB copolymers. Further, in vivo tolerability study was performed following topical instillation and intravitreal injection. Results demonstrated excellent tolerability without showing any signs of inflammation or cataract. Results revealed that PB copolymer based formulation can be used as a platform for the treatment of posterior segment ocular diseases.

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ASHIM MITRA: No financial relationships to disclose

P250

HYPOXIA AND RETROMER AS POTENTIAL REGULATORS OF POLARIZED AMYLOID PRECURSOR PROTEIN EXPRESSION IN RPE CELLS

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NORTH CAROLINA STATE UNIVERSITY, MOLECULAR BIOMEDICAL SCIENCES

Amyloid precursor protein (APP) is a ubiquitously expressed protein that produces amyloid-beta (A β), a key component of Alzheimer's disease which is also found in drusen present in age-related macular degeneration (AMD). Retromer, an intracellular retrograde transport complex, shuttles APP away from key cleavage enzymes, limiting the production of A β . Retromer regulates the polarized movement of APP from the trans Golgi network to apical or basolateral surfaces for secretion. Polarized secretion of APP by retinal pigmented epithelial (RPE) cells under normoxic and hypoxic conditions has not been previously studied. Our current studies have found that oxygen levels do indeed control polarized secretion of APP in RPE cells. Specifically, using our unique cell culture system of polarized, tight junctional RPE cells we determined that 4.5 times more APP was secreted basolaterally versus apically. Under hypoxic conditions (0.5% oxygen) APP secretion was decreased in both the apical (94%) and basolateral (72%) direction; while still maintaining a 4 fold higher level of APP secretion in the basolateral versus apical direction. An

additional novel finding is hypoxia's effect on retromer levels. Hypoxia decreases retromer levels in polarized RPE cell lysates by 89%. Significantly, when retromer was knocked down with siRNA in non-polarized RPE cells, APP secretion is reduced an average of 38% in both normoxic and hypoxic conditions. We are currently determining if the effect of hypoxia on retromer causes alteration of the polarized secretion of APP. As hypoxia is a common pathology of the retina, understanding how this condition affects protein processing, polarized localization, and secretion is vital to our comprehension of RPE pathophysiology.

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Disclosures:

PHILIP MZYK: No financial relationships to disclose

P251

VISUALIZATION OF IRON REGULATED CHANGES IN FERROPORTIN EXPRESSION AND SUBCELLULAR LOCALIZATION IN CULTURED CANINE RPE CELLS

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Ferroportin (Fpn) is the only documented iron exporter in mammalian cells, and is thus essential for cellular iron efflux. The presence of excess iron in ocular pathologies such as AMD may reflect a defect in the ability of the cell to remove iron via ferroportin. Some cells of the neural retina and the retinal pigmented epithelial (RPE) cells have been shown to contain Fpn, but the subcellular localization and role of Fpn in iron efflux in cultured ocular cells is not well documented. Using immunofluorescence in primary canine RPE cells, we localized Fpn to the plasma membrane, where it would be expected to function in removal of excess iron from the cell, and also to subcellular organelles, suggesting that Fpn may also function in the redistribution of iron between different membrane-limited compartments of the cell. To better characterize the normal sorting pathway and function of Fpn in a RPE culture system that better reflects the *in vivo* condition, we examined Fpn expression using a tight-junctional, polarized RPE cell culture system. In these polarized RPE cells, we observed Fpn localization in both apical and basolateral membranes, suggesting that iron may exit these cells from both membrane domains. In order to visualize those changes in Fpn localization that may occur during a relatively short period of time, we transfected RPE with a construct encoding a Fpn-GFP fusion protein to track those changes in Fpn expression and localization that occur at the earliest time points after treating cells with exogenous iron. When RPE were subjected to iron overload, we observed a significant increase in Fpn-GFP expression at 6-12 hours post treatment, accompanied by subsequent movement of Fpn-GFP to the plasma membrane. Studying the dynamics of Fpn expression and movement in real time will be useful to further characterize iron efflux in RPE cells.

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OVEREXPRESSION OF HTRA1 AND EXPOSURE TO CIGARETTE SMOKE EVOKE CHOROIDAL NEOVASCULARIZATION AND RETINAL DEPOSITS IN AGED MICE

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Purpose: Recent genome wide association (GWAS) studies of age-related macular degeneration (AMD) have revealed associations with over 30 loci, most significantly for ARMS2/HTRA1 on chromosome 10q26. To determine the function of these genes in the choroid and the retina, transgenic (Tg) mice ubiquitously overexpressing ARMS2, ARMS2 (A69S), and Htra1 cDNA were developed and observed for fundus changes and pathological changes at over 12 month period after birth. We also examined the effects of mainstream cigarette smoke for these Tg mice to evaluate the progression of the disease by environmental risk factor.

Methods: Chicken actin promoter (CAG) was used to drive mouse *Htra1* and human *ARMS2*, *ARMS2 (A69S)* expression in entire mouse body and maintained for one year. Fundus observation was performed by Spectralis HRA+OCT. The eyes were embedded and sectioned for H&E staining and immunohistochemistry. Smoking exposure to mice was performed using mainstream smoking chamber (INH06-CIGR02A, MIPS) for 30 min/day and 5 days/week for 12 weeks. After 12 weeks, fundus photo and pathological analysis were performed.

Results: Approximately 18.2% of *Htra1* Tg mice at 12 month exhibited choroidal neovascularization (CNV) by OCT and positive immunostaining with anti-CD31 and anti-fibronectin antibodies. Furthermore, *Htra1* Tg mice also showed Bruch's membrane damage by elastic van Gieson (EVG) staining. No change was observed for *ARMS2* or *ARMS2 (A69S)* Tg mice. Smoking exposure for three month enhanced CNV by approximately 7.7% for control and 20% for Tg mice, but *ARMS2* Tg mice remain normal. In addition, abnormal deposit was observed between photoreceptor and retinal pigment epithelium exclusively for *Htra1* Tg mice exposed to smoking.

Conclusions: *Htra1* overexpression or smoking can independently evoke CNV and *Htra1* is a strong risk factor for wet AMD, but not all the mice tested were affected, suggesting that CNV is developed based on multiple risk factors.

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A CELLULAR DISEASE MODEL SYSTEM FOR AUTOSOMAL-RECESSIVE BESTROPHINOPATHY: THE CREATION OF IPS-RPE FROM A PATIENT WITH PREMATURE STOP MUTATION (P.R200X)

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Autosomal-recessive bestrophinopathy (ARB) is a distinct inherited bestrophinopathy that is caused by a nonsense mutations in the bestrophin 1 (BEST1), a protein located in the retinal pigment epithelium (RPE). The p.R200X mutation is a premature stop mutation that causes alterations in the RPE and subretinal deposits in the macular area. Patients with this mutation have an absent EOG light rise and reduced ERGs, presenting with central vision loss early in life. Currently, patients with Bestrophinopathies, such as ARB, do not have any available treatments and visual loss cannot be prevented. Resolving the exact location and function of the BEST1 in RPE cells is an essential step in identifying viable therapeutics for these patient groups. Our laboratory has previously demonstrated that RPE can be produced from human embryonic (hES) and induced pluripotent stem (iPS) cells. Thus, in order to better understand the role of BEST1 in RPE cells we have created iPS cells from the p.R200X patient fibroblasts by reprogramming with episomal vectors (c-myc, Klf4, Lin28, Oct4, Sox2). iPS colonies were isolated, expanded and differentiated into RPE by spontaneous differentiation method. After approximately 6 weeks pigmented foci were purified by manual dissection and cells were seeded to encourage monolayer formation. Patient iPS and iPS-derived RPE cells were assessed by standard molecular and cellular protocols, including immunocytochemistry and PCR, in comparison to control cells. In future we aim to use these cells to model patient disease in a dish and to identify potential treatments.

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NUMBER AND LOCATION OF LESIONS FOR THE OPTIMIZATION OF THE EXAMINATION INTERVAL IN DIABETIC RETINOPATHY

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A recent study concluded that it is possible to construct a model for optimising the examination interval during screening for diabetic retinopathy in low-risk patients¹. However, the model fails to predict the interval for patients in whom the primary assessment recommended a short screening interval, suggesting that more confounders should be identified and included.

Two sets of fundus photographs, one where the result of the model and recommended interval are concordant and one where they are discordant, were analysed. The number of different lesions and their centre position with regards to the areas defined on a previous study were stored in an array of features associated with the relative fundus photo. Considering the concordance and discordance on the interval as the two possible values of a dependent variable in a classification problem, a feature extraction and a 10-fold cross validation were performed to assess their significance. The feature selection chose as the two best isolated features the number and the percentage of small haemorrhages in two different areas respectively. The 10-fold cross validation performed extracting the first two ranked features only resulted in the correct classification of 64% of the instances. The ranking of the feature selection matched the clinicians' experience while the percentage of correct classifications suggested that the model would benefit from the information held in the location and number of small haemorrhages showing on fundus photography.

¹Mehlsen et al., *Acta Ophthalmol. Scand.* 90(109-114), 2012.
bHove et al., *Acta Ophthalmol. Scand.* 82(679-685), 2004.

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P255

FETAL HEMOGLOBIN INDUCTION BY MONOMETHYLFUMARATE: RELEVANCE TO PREVENTION AND TREATMENT OF RETINOPATHY IN SICKLE CELL DISEASE (SCD)

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Sickle cell retinopathy (SR) is a most debilitating common complication of SCD. Abnormal hemoglobin (HbS) polymerization in RBCs and consequent vascular dysfunction are primary causative factors; however, clinical and experimental studies support the critical involvement of other cellular/molecular factors. Retinal pigment epithelial (RPE) cells were reported recently to produce Hb; this phenomenon has not been evaluated in the context of retinal pathology in SCD. Here, using ARPE-19, primary RPE cells from HbAA (normal)- and HbSS (sickle)-expressing Townes humanized mice, KU812 and primary human erythroid progenitors we (a) evaluated globin gene expression/Hb production in normal and sickle mouse retina, and (b) validated monomethylfumarate (MMF) as an effective inducer of γ -globin/fetal Hb (HbF) production in retinal and erythroid cells and therefore, as a potential therapy

for preventing/treating retinal AND systemic complications of SCD. RPE cells were treated with MMF (0 – 1000 mM; 24 h). Globin gene expression/HbF production was analyzed by qPCR, western blotting, FACS and immunofluorescence. Cells treated with hydroxyurea (HU), at present the only FDA-approved HbF-inducing drug, were included for comparative purposes. Parallel studies were performed using erythroid cells. RPE cells express α -, β - and γ -globin mRNA and synthesize normal (ARPE-19 and HbAA primary RPE) and sickle (HbSS primary RPE) Hb. MMF induced, in a time- and dose-dependent manner, γ -globin expression/HbF production in all cell types evaluated, and in retina following its delivery intravitreally to live mice. The induction of HbF by MMF was 2-3-fold higher than that by HU. Our finding that MMF induces γ -globin expression and HbF production is novel and supports the possible use of this compound in SCD/SR. The ready availability of FDA-approved formulations in which MMF is the main bioactive ingredient enhances greatly the translational potential of this study.

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P256

HISTOPATHOLOGY OF THE RETINA IN EYES FROM PATIENTS WITH BEST DISEASE CAUSED BY VMD2 MUTATIONS

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The histopathology of donor eyes (an 85 year old female [donor 1] and a 65 year old male [donor 2]) from patients with Best Disease (BD) caused by Asn296His and Ile201Thr VMD2 mutations was evaluated. Macroscopic, SLO and OCT imaging techniques were used to examine globes. Peripheral and perifoveal retinal areas were processed for electron microscopy and immunocytochemistry using cell-specific antibodies. Age-similar (61-88 year old) normal eyes were used as controls. DNA was taken from donor blood samples. Sequence analysis of the entire VMD2 coding region was done. DNA analysis of donor 1 showed an Asn296His VMD2 mutation and donor 2 showed an Ile201Thr VMD2 mutation at exon 5. Histology showed a distinct ganglion cell layer (GCL), inner nuclear layer (INL), outer nuclear layer (ONL), RPE and choriocapillaris (CC) in the periphery of each Best Disease donor. Prominent GCL and INL were evident in the perifoveal region of donor 1. The perifoveal region of donor 2 had definite GCL, INL, ONL and a robust RPE and CC. Cells labeled with cone opsin and arrestin antibodies were present in the macula, but mostly absent in the retina adjacent to a fibrovascular scar of donor 1. Rhodopsin labeling was detected in the perifoveal region but not in the fibrovascular scar area of donor 1. In donor 1, autofluorescent material in the perifoveal region was significantly reduced in areas where

the RPE was still present. The histopathology of the retina from an individual with *VMD2* Asn296His mutation (donor 1) showed a highly degenerated perifoveal retina. The retina in the individual with an Ile201Thr *VMD2* mutation (donor 2) showed normal perifoveal morphology with preservation of cones and rods in the periphery.

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MARY RAYBORN: No financial relationships to disclose

P257

A COMPARISON OF PHAGOCYTOSIS IN FETAL VS. STEM CELL DERIVED RPE CELLS

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The Retinal Pigmented Epithelium (RPE) is required to maintain the health of photoreceptors. The RPE is highly polarized and this polarization is essential to critical functions such as photoreceptor outer segment (OS) phagocytosis. Methods to derive RPE cells from human embryonic stem (ES) cells have been established, and monolayers of ES-derived RPE have been proposed as a therapy for patients with age-related Macular Degeneration. We are interested in the competency of RPE cells derived from ES cells to perform OS phagocytosis. The "gold standard" for evaluating RPE phagocytosis in vitro utilizes monolayers derived from postmortem human fetal eyes. Specifically, the purpose of our project was a head to head comparison of photoreceptor OS binding and phagocytosis in cultured monolayers of fetal-derived (FD) RPE to monolayers of RPE derived from the H9 ES cell line. Bovine OS were isolated from postmortem eyes and fluorescently labeled. FITC labeled Bovine OS were pipetted in equal concentrations into the polarized cultures and incubated for 1, 6, 11, and 16 hours. Trypan blue was used to quench external FITC before the wells were cut, fixed, and plated for viewing under a confocal fluorescent microscope. Our preliminary results show an increase in apical binding with significantly more FITC intensity at the later timepoints of 11 and 16 hrs. However, no significant difference was found between the cell surface adherence of OS in the FD versus the ES-derived RPE at the 1, 6, 11, and 16 hour timepoints. Similarly, no significant difference was found in phagocytosis between the FD versus ES-derived RPE at any of the timepoints based on area and pixel intensity of FITC staining. These results suggest that H9-derived RPE phagocytose OS as efficiently as those derived from human fetal eyes.

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P258

DIFFERENTIALLY EXPRESSED EXTRACELLULAR RNA IN SERUM OF AMD PATIENTS

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The extracellular RNA (ex-RNA) has recently been identified as novel biomarkers in a number of important chronic diseases. The purpose of our study was to identify differentially expressed serum exRNA that could be potentially used as early diagnostic biomarkers for age-related macular degeneration (AMD) patients. Serum samples of 30 patients from 3 different groups were collected: Group 1, age-matched normals; Group 2, dry AMD; Group 3, new wet AMD. RNA was isolated using modified Qiagen microRNeasy procedure, and quantitated on BioAnalyzer's small RNA chips. Microarray miRNA analysis of serum samples was performed on 4 samples from each group. Microarray data was analyzed using GeneSpring software and Ingenuity Pathway Analysis software. Confirmatory quantitative real-time PCR (qPCR) was performed on the select set of biomarker candidates for the full sample set of each group. Many statistically significant ($p < 0.01$) miRNA differences were seen between the 3 groups involving both increased and decreased levels. The miRNAs showing largest dysregulations are listed in parenthesis. Dry AMD vs. control had 10 (miR-661 up, miR-3121 down), while wet AMD vs. control had 9 (miR-4258 up, miR-889 down). Ingenuity pathway analysis identified pathways that might be affected by dysregulated ex-RNAs between AMD patients and normal, i.e. inflammatory and angiogenic pathways. The exRNA levels that were significantly altered between the groups tested may offer diagnostic and therapeutic benefits.

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P259

A QUANTITATIVE STRUCTURAL COMPARISON OF THE MOUSE CENTRAL RETINA AND THE HUMAN MACULA: IS THE MOUSE AN APPROPRIATE MODEL FOR MACULAR DEGENERATION?

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The most common retinal degenerations concern the macula, and mice do not have a macula *per se*. So, how relevant is the mouse as a model for macular degeneration? In the present study, a quantitative comparison between the central mouse retina and the human macula was made, focusing on key structural parameters that are likely to be important with

regard to predisposing the macula to stresses leading to degeneration. The photoreceptor density in mouse retinas was found to be slightly higher in the central area of the retina and declined towards the periphery. RPE cells in the central retina were almost twice the size as the ones in the periphery, so that the number of photoreceptor cells per RPE cell was significantly higher in the central retina. Bruch's membrane was found to be 1.5 times thinner in the central retina than in the periphery. Comparing these data with those reported for the human retina, some similarities are evident. Photoreceptor cell density is higher in the central retina than in the periphery, although the difference is greater in the human retina, and the density is higher in all regions of the mouse retina than in the human retina. In both mouse and human retinas, Bruch's membrane is thinner in the central region. In contrast to the mouse RPE, human RPE cells are significantly smaller in the center of the retina. Nevertheless, the number of photoreceptor cells per RPE cell is higher in the centers of both retinas. Therefore, while there are quantitative differences, both mouse and human retinas have similar center-periphery trends, with respect to some structural characteristics that are likely to factor in susceptibility to pathogenesis related to macular degeneration.

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P260

GENE PROFILING OF POSTNATAL MFRPRD6 MUTANT EYES REVEALS DIFFERENTIAL ACCUMULATION OF PRSS56 MRNA

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Mutations in the membrane frizzled-related protein (*MFRP*/*Mfrp*) gene, specifically expressed in the retinal pigment epithelium (RPE) and ciliary body, cause nanophthalmia or posterior microphthalmia with retinitis pigmentosa in humans and photoreceptor degeneration in *Mfrp*^{rd6} mice. To better understand MFRP protein function, a microarray analysis was performed on eyes of homozygous *Mfrp*^{rd6} and C57BL/6J mice at postnatal days (P) 0 and P14, prior to photoreceptor loss. Data and pathway analysis revealed no changes at P0 but significant differences in RPE and retina-specific transcripts at P14, suggesting a postnatal influence of the *Mfrp*^{rd6} allele. *Prss56*, which encodes a trypsin-like serine peptidase, was significantly elevated in *Mfrp*^{rd6} mice. Validation by qRT-PCR indicated a 3.5-, 14- and 70-fold accumulation of *Prss56* transcripts relative to controls at P7, P14 and P21, respectively. This trend was not observed in other RPE or photoreceptor mutant mouse models with similar disease progression, suggesting that *Prss56* upregulation is a specific attribute of *Mfrp* deficiency. Interestingly, like mutations in *MFRP*, humans with a disruption in *PRSS56* have been shown to develop autosomal recessive posterior microphthalmos (Gal et al. 2011; Nair et al. 2011, Said et al. 2013), angle closure glaucoma (Nair et al. 2011; Jiang et

al. 2013), nanophthalmos (Orr et al. 2011, Said et al. 2013) and axial length and refractive error in extremely large GWA studies (Verhoeven et al. 2013; Kiefer et al. 2013). From our analysis of both *Mfrp*^{rd6} and *Prss56* mutants, it is likely that MFRP acts upstream of PRSS56. The link between *Mfrp* deficiency and *Prss56* upregulation, together with the genetic association of human *MFRP* or *PRSS56* variants and ocular size, raises the possibility that these genes are part of a regulatory network influencing posterior eye growth.

P261

RECOVERY OF TRANSEPITHELIAL ELECTRIC RESISTANCE FROM OXIDATIVE INJURY BY HUMANIN AND ROLE OF MITOCHONDRIA IN PROTECTION OF RPE CELLS

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Recent studies showed that Humanin (HN), a 24-amino acid peptide, has a neuroprotective role in multiple cell culture and animal models. However, its role in age-related macular degeneration is unknown. We investigated a) the expression of HN in hRPE cells, b) its protective role against oxidative stress (OS)-induced cell death, c) its role in mitochondrial respiration, and d) its role in restoration of transepithelial electric resistance (TER) in RPE monolayers. HN localization in RPE cells and polarized RPE monolayers was assessed by confocal microscopy. To study the protective effect of HN, primary hRPE cells were co-treated with varying doses of HN (0.5-10ug/ml) and 150 μM of tert-Butyl hydroperoxide (tBH) for 24 h. Mitochondrial respiration was measured in RPE cells using XF96 analyzer (Seahorse Bioscience Inc, MA). RPE cell death and caspase-3 activation induced by tBH were studied by TUNEL staining and immunoblot analysis. OS-induced change in TER was studied in polarized RPE monolayers with and without HN co-treatment using cellZscope (nanoAnalytics, Germany). Confocal studies showed a prominent expression of HN in the cytoplasm and nucleus of hRPE cells. In the cytoplasm, HN co-localized with mitochondria. Human polarized RPE monolayers did not exhibit selective vectorial expression. Mitochondrial respiration was decreased with OS (p<0.05 vs control) while HN co-treatment upregulated mitochondrial respiration (p<0.01 vs tBH treated cells). HN protected RPE cells from OS-induced cell death and prevented caspase-3 activation. In polarized RPE cells, HN treatment restored OS-induced decrease in TER. Thus, we conclude that HN protects RPE cells against OS-induced cell death, and restores mitochondrial function and TER against oxidant stress. These data suggest promising therapeutic potential for HN in the prevention and/or treatment of AMD.

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IRB Status: Approved

Disclosures:

SREEKUMAR PARAMESWARAN: No financial relationships to disclose

CONNECTIVE TISSUE GROWTH FACTOR IS A KEY REGULATOR OF OXYGEN-INDUCED RETINOPATHY

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The matricellular protein connective tissue growth factor (CTGF), known also as CCN2, is an inducible immediate-early gene-encoded, extracellular matrix protein which appears in the pericellular matrix during development and in angio-proliferative disorders such as ischemic retinopathies. The CTGF gene is essential for viability as CTGF-deficient mice exhibited severe deficiencies in skeletogenesis and new blood vessel formation causing embryonic lethality. CTGF promoter-GFP reporter mice were used to determine the tissue distribution of CTGF during retinal vessel development in mice. CTGF promoter-driven GFP, which recapitulates endogenous CTGF expression in mice, showed a dynamic but transient expression of the transgene with maximum expression during the formation of the primary

and secondary capillary plexuses. Endothelial cells and pericytes were major sources of CTGF. CTGF was abnormally increased and localized within neovascular tufts in the retina from mice subjected to oxygen-induced retinopathy (OIR). Ectopic expression of the CTGF gene through lentivirus-mediated gene transfer further exacerbated neovascularization in the retina while lentivirus-mediated loss-of-function or -suppression of CTGF significantly reduced ischemia-induced neovascular growth in mice. The neovascular effects of CTGF were mediated, at least in part, through increased expression and activity of matrix metalloproteinase (MMP)-2. In cultured cells, CTGF activated MMP-2 promoter through increased expression and tethering of the p53 transcription factor to a highly conserved p53 binding sequence within the MMP-2 promoter. Concordantly, the neovascular effects of CTGF were suppressed by p53 inhibition which culminated into reduced enrichment of the MMP-2 promoter with p53 and decreased MMP-2 gene expression. These data identified new gene targets and downstream effectors of CTGF and provided the rationale basis for targeting the p53 pathway to curtail the effects of CTGF on neovessel formation associated with ischemic retinopathy.

IRB Status: Not provided

Disclosures:

BRAHIM CHAQOUR: No financial relationships to disclose

Anterior/Posterior Segment

Wednesday Viewing: 10:00 – 10:30
12:00 – 13:00

Thursday Viewing: 10:00 – 10:30
11:45 – 13:00

Session with Authors: 15:00 – 16:30

P301

FROM COLOBOMA TO CYCLOPIA: DECIPHERING THE MORPHOGENETIC ROLE OF THE NEURAL CREST ON EYE DEVELOPMENT

SOUFIEN SGHARI, Sophie Creuzet

INSTITUT ALFRED FESSARD DE NEUROBIOLOGIE

The cephalic neural crest (CNC) is a transient structure in embryo, which contributes to ocular development by providing a highly plastic and pluripotent mesenchyme, which generates many periocular derivatives.

In the anterior segment, two main areas of CNC differentiation can be distinguished: an ocular one comprising the iris, the ciliary process and the cornea that delineate the anterior optic chamber, and a peri-ocular one including the nictitating membrane and eyelids. Due to the “pleiotropy” of the CNC contribution to normal eye development, the dysfunctions of the CNC can encompass a multiplicity of pathogenic conditions. Many congenital malformations in ocular development and pathogenic conditions stem from perturbations of CNC derivatives. However, the signaling pathways required for the specification of the CNC-derived ocular mesenchyme and, in turn, the morphogenetic role of CNC on ocular development remains to be documented.

By combining classical embryological approaches (microsurgery), long-term cell lineage tracing (xeno-transplantation), and in ovo electroporation for functional studies, our work aims at identifying the molecular pathways responsible for the morphogenetic role of the CNC in ocular development. We have already identified some signaling molecules involved in the growth and specification of the retina. Our results show that some defaults resulting from the absence of CNC primarily stem from a defective specification of the optic stalk and the polarity of the neural epithelium at this level. Our present work provides novel insights into epithelio-mesenchymal cross-talks involved in eye development the deregulation of which account for the multiplicity of ocular malformations.

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SOUFIEN SGHARI: No financial relationships to disclose

P302

REDUCED EFFECTIVE FILTRATION AREA FOR AQUEOUS OUTFLOW IS CORRELATED WITH DECREASED OUTFLOW FACILITY IN SPHINGOSINE-1-PHOSPHATE TREATED BOVINE EYES

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Previous studies in both porcine and bovine eyes revealed Sphingosine-1-phosphate (S1P) reduces outflow facility (C); however, morphological correlations to this reduction have not been clarified. We hypothesize this decreased C is a result of reduced effective filtration area (EFA), which results from increased connectivity of juxtacanalicular tissue (JCT) and inner wall (IW) cells of aqueous plexus (AP). Seven pairs of freshly enucleated bovine eyes were all first perfused at 15mmHg for 30min to establish a baseline C, and then additionally perfused for 2hrs with either 5μM S1P or GPBS. Eyes were then exchanged and perfused with fluorescent microspheres (0.002%, 0.5μm) to trace outflow patterns followed by perfusion fixation. All pairs were dissected into frontal sections for confocal microscopy. Effective filtration length was measured along IW/JCT area and calculated [PEFL = filtration length (FL) / total length (TL)]. All tissue was processed for light microscopy and sectioned to measure IW/JCT separation (SL) and percent separation length measured [PSL = SL / TL]. Two-tailed Student's t-test was used for statistical analysis. S1P and control groups both had significantly increased C compared to baseline (p=0.04; p=0.002). While both groups also showed washout, the percent increase in C by S1P (37±9%) was significantly lower than that of controls (143±55%; p<0.01). Additionally, S1P (19±3%) caused a significant reduction in PEFL compared to controls (51±1%; p<0.01). Interestingly, a significant positive correlation was found between PEFL and % increase in C, but no difference in PSL was seen between both groups (p=0.68). While our results confirm our hypothesis that decreased C by S1P is due to reduced EFA in bovine eyes, no morphological association was found between increased IW/JCT connectivity and C. Therefore, further examination of morphology is required to better understand what structural changes account for decreased EFA by S1P.

IRB Status: Approved

Disclosures:

LAIYIN MA: No financial relationships to disclose

BLOCKADE OF ADENOSINE A2A RECEPTOR PROTECTS RETINAL GANGLION CELLS AGAINST ELEVATED PRESSURE-INDUCED CELL DEATH BY CONTROLLING NEUROINFLAMMATION

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IBILI, FACULTY OF MEDICINE, UNIVERSITY OF COIMBRA, PORTUGAL

Glaucoma is the second leading cause of blindness worldwide, being characterized by a progressive optic nerve damage and loss of retinal ganglion cells (RGCs). The etiology of glaucoma is unknown, and despite elevated intraocular pressure (IOP) being the main risk factor, the exact mechanisms responsible for RGC degeneration in glaucoma remain unknown. The degeneration of RGCs in glaucoma is accompanied by an increased inflammatory response involving retinal microglial cells. In the brain, the blockade of adenosine A2A receptors (A2AR) confers robust neuroprotection in several neurodegenerative disease models and controls neuroinflammation and microglia activation. The aim of this work was to evaluate the potential protective effect of A2AR blockade against retinal neuronal cell death induced by elevated hydrostatic pressure (EHP), and test if this protective effect might be mediated by inhibition of retinal neuroinflammation. Cultured retinal explants were pretreated with 50 nM SCH58261 (selective A2AR antagonist) and challenged with elevated hydrostatic pressure (EHP; 70 mmHg above atmospheric pressure), mimicking elevated IOP. EHP increased the expression of A2AR in microglial (CD11b-immunoreactive) cells present in the retinal ganglion cell layer and increased the extracellular levels of ATP. Blockade of A2AR prevented the alterations in microglia morphology as well as the increased expression and release of inflammatory mediators induced by EHP. Similar results were obtained by removing extracellular adenosine with adenosine deaminase (2 U/mL). Moreover, the EHP-induced decrease in the number of RGCs (Brn3a-immunoreactive cells) was prevented either by A2AR blockade or by neutralizing antibodies against TNF and IL-1 β . Our results show that A2AR blockade confers neuroprotection to RGCs by controlling retinal neuroinflammation induced by EHP, indicating that A2AR antagonists have a therapeutic potential in the treatment of glaucoma.

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Disclosures:

MARIA HELENA MADEIRA: No financial relationships to disclose

GENETICALLY DISSECTING THE PRIMARY SITE OF PATHOGENESIS IN COL4A1 MEDIATED ANTERIOR SEGMENT DYSGENESIS

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Anterior segment dysgenesis (ASD) is a spectrum of disorders affecting the development of anterior structures of the eye that can lead to the development of glaucoma and vision loss. Mutations in a basement membrane component, collagen type IV alpha1 (COL4A1) cause ASD in mice and humans. Because of the widespread distribution of COL4A1 in all ocular membranes and the involvement of multiple tissues of the anterior segment in ASD, dissecting the primary site of pathogenesis can be difficult. Here, we use conditional expression of mutant COL4A1 to determine the primary location of insult in COL4A1-mediated ASD. We developed a conditional allele with *LoxP* sites flanking exon 41 of *Col4a1* (*Col4a1 flex41*) that recreate the *Col4a1 Δex41* allele known to cause severe ASD affecting all anterior segment structures in mice. Four tissue-specific CRE recombinase strains, *MLR10-Cre* (lens specific expression), *Wnt1-Cre* (neural crest derived periorbital mesenchyme expression), *Tie2-Cre* (vascular endothelial cell expression) and *Pdgfrb-Cre* (pericyte expression), were crossed with *Col4a1 +/flex41* mice to generate corresponding tissue-specific mutants. In addition, *Actb-Cre* mice (ubiquitous expression) were used to validate the *Col4a1 flex41* allele for CRE-mediated excision. Slit-lamp and histological examinations were performed to assess the extent of ASD. We found that while *Col4a1 +/flex41; Actb-Cre* mice recapitulated the complete ASD spectrum observed in *Col4a1 +/Δex41* mice, only *Col4a1 +/flex41; MLR10-Cre* mice developed mild ASD affecting only the lens. Conditional expression of mutant COL4A1 in other tissues did not cause any defects in the anterior segment. Our results indicate in tissues other than the lens, a non-cell autonomous mechanism is needed to cause the disease and that effect of mutant COL4A1 expression in one tissue may be compensated by expression of wildtype COL4A1 from other tissues.

IRB Status: None

Disclosures:

MAO MAO: No financial relationships to disclose

NORMAL AND GLAUCOMATOUS HUMAN LAMINA CRIBROSA AND TRABECULAR MESHWORK CELL BEHAVIOURS AS DETERMINED BY RIGIDITY OF THE SURROUNDING EXTRACELLULAR MATRIX

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The purpose of this research project was to assess the differential ability of normal and glaucomatous human Lamini Cribrosa (LC) and Trabecular Meshwork (TM) cells to interact with their surrounding extracellular matrix. Human LC and TM cells were grown on a range of cellular matrices of varied stiffness. The response of cells to differing environments was assessed by analysis of cell movement, growth and contractility processes. An analysis of the in-vitro migratory, proliferative and contractile capacity of trabecular meshwork and lamina cribrosa cells obtained from normal and glaucoma patient donors was

performed by means of scratch wound assays, indirect immunofluorescence and quantitative PCR analysis of monolayer and 3D culture conditions. Preliminary data shows the ability of the stiffening-ECM to induce phenotypic changes in normal TM cells such that they adopt an abnormal (fibroblastic) morphology. We have found cellular processes of migration, proliferation and contractility to be altered in the glaucomatous disease state. Furthermore, normal and glaucomatous TM cells seeded onto a laminin-rich ECM display disparate patterns of cellular network organisation. Glaucomatous TM cells have a higher proliferative index than normal TM cells, as judged by Ki67 staining. TM cells treated with the pro-fibrotic cytokine Transforming Growth Factor beta (TGF-beta) express elevated levels of fibrotic markers (e.g. snail, thrombospondin (TSP1), vimentin; $P < 0.05$). We propose TGF-beta and one of its activators TSP1 as major factors which drive abnormal cell behaviour, and consequently, irreversible disease progression. We believe that a 'matrix-stiffening model' proves a reliable tool with which to detect the differential responses of normal versus glaucomatous cells to the extracellular environment. This research project has enabled the definition of normal versus disease cell behaviour on a molecular level.

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SARA MCNALLY: No financial relationships to disclose

P306

SENSITIVITY OF THE *NEE* ALLELE TO GENETIC BACKGROUND

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The *nee* mouse strain, harboring a recessive mutation in *Sh3pxd2b*, exhibits severe early onset glaucoma with elevated intraocular pressure, optic nerve excavation, and axon loss. The closed angle in *nee*-mutant mice results from the failure of the iris and cornea to separate during development with apparent aplasia of the trabecular meshwork and Schlemm's Canal. To ascertain the effect of genetic background on ocular phenotypes of the *nee* allele, we first produced an N10 congenic strain transferring the *nee* mutation from an infrequently used B10 genetic background onto a B6 genetic background. Indistinguishable from the B10-*nee* strain of mice, homozygosity of the *nee* mutation on the B6 background resulted in congenital iridocorneal adhesion as well as cloudy corneas and enlarged anterior chambers by 3 months of age. Subsequently, we initiated an intercross between B6.*nee* and wild-derived inbred CAST/EiJ mice to produce an F2 population. In contrast to the B6.*nee* strain, the iridocorneal angle phenotypes of *nee*-mutant F2 progeny display substantial variable expressivity, with 49/64 *nee*-mutant mice having no indication of iridocorneal angle developmental abnormalities. The genetic complexity of phenotypic variability among *nee*-mutant F2 progeny was assessed with interval mapping for binary traits using R/qtl. Preliminary results identified a significant locus on chromosome 16 (LOD 4.27), indicating the presence of a genetic modifier capable of influencing *nee*-mediated ocular phenotypes. The B6.*nee*

congenic mouse, which recapitulates phenotypes published using the B10 background, will be a valuable resource for future studies of glaucoma. In addition, the overt ocular phenotypes of B6-*nee* mice, which are altered in the F2 mixed genetic background, provide the opportunity to identify modifier genes contributing to pathological molecular pathways for glaucoma. Future studies will focus on mapping the gene(s) responsible for the variable expressivity in angle development and testing the role of *Sh3pxd2b* in adult mice.

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Disclosures:

KACIE MEYER: No financial relationships to disclose

P307

NORRIN INHIBITS THE DEVELOPMENT OF GLAUCOMA IN DBA/2J MICE

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Purpose: Norrin, a secreted signaling molecule, protects retinal ganglion cells (RGC) against an excitotoxic NMDA-mediated damage via an activation of the Wnt/ β -catenin signaling pathway. Here we investigated if Norrin has similar neuroprotective properties on chronic RGC death in glaucoma. In addition, the roles of IGF-1 expression and AKT pathway activation in Norrin-mediated neuroprotection were investigated.

Methods: Transgenic mice with an overexpression of Norrin in cells derived from the optic cup under the specific control of the alpha enhancer element of the Pax6 promoter (Pax6-Norrin) were generated in the genetic background of DBA/2J mice. Intraocular pressure (IOP) was measured, and morphological changes were investigated by light microscopy. Retinal expression of IGF-1 and phosphorylation of AKT was analyzed by real-time RT-PCR, western blotting and immunohistochemistry.

Results: In Pax6-Norrin / DBA/2J mice, a moderate expression of Norrin mRNA and an activation of the Wnt/ β -catenin pathway were detected in the retina. In DBA/2J mice, an increased intraocular pressure (IOP) with a maximum of 17.8 ± 1.2 mmHg at the age of 9 months was detected. In contrast, in 9-month old Pax6-Norrin / DBA/2J littermates, IOP was significantly lower (12.7 ± 0.8 mmHg). Moreover, by light microscopy and semi-quantitative analysis of the trabecular meshwork, a less severe damage of the aqueous humor outflow tissues was observed in Pax6-Norrin / DBA/2J mice than in DBA/2J littermates. The quantification of RGC axons in the optic nerves showed significantly more axons in Pax6-Norrin / DBA/2J mice compared to DBA/2J littermates. In addition, levels of IGF-1 mRNA and pAKT were significantly increased in retinae of Pax6-Norrin / DBA/2J mice compared to DBA/2J controls.

Conclusions: Transgenic overexpression of Norrin reduces glaucomatous damage in DBA/2J mice most likely by IOP reduction and an increased expression of IGF-1 which in turn enhances AKT phosphorylation.

IRB Status: None

Disclosures:

ANDREAS OHLMANN: No financial relationships to disclose

P308

ADENOSINE A2A RECEPTOR BLOCKADE AND CAFFEINE MODULATE RETINAL NEUROINFLAMMATION FOLLOWING ISCHEMIA-REPERFUSION INJURY

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Retinal ischemia-reperfusion (I-R) injury contributes to several ocular diseases including glaucoma, central retinal artery occlusion, ischemic optic neuropathy, and diabetic retinopathy. Activation of microglia/macrophages has been observed in I-R injury, and it has been associated with neuronal injury and death. In the brain, the blocking of adenosine A2A receptor (A2AR) prevents neurodegeneration possibly by modulating the release of noxious factors by activated microglia. Evidence also suggests that caffeine, a non-selective adenosine receptor antagonist, attenuates inflammatory responses and affords protection upon CNS injury. The aim of this work was to investigate whether A2AR antagonist or caffeine intake prevent retinal neuroinflammation and cell death induced by retinal I-R injury. Wistar rats were subjected to 60 min of pressure-induced retinal ischemia. Rats were treated with A2AR antagonist (SCH58261; 100 nM, 5 µl intravitreal injection) or caffeine (1 g/l in the drinking water) prior to I-R. At 24 h or 7 days post-ischemia we assessed cellular inflammation, retinal gene expression, and DNA fragmentation. Blockade of A2AR decreased the effects of I-R injury on microglia reactivity and on the expression of IL-1β, TNF and GFAP. At 24h post-ischemia, caffeine exacerbated microglia reactivity, without significant changes in the levels of TNF, IL-1β and IL-6. In addition, caffeine significantly enhanced the number of TUNEL-positive cells induced by I-R injury. However, at 7 days post-ischemia we found that caffeine decreased the effects of I-R on the number of active microglia, on the levels of TNF and IL-1β, and on the production of reactive oxygen species. Moreover, the number of TUNEL-positive cells in the retina was significantly decreased in caffeine-treated animals. These results suggest that A2AR blockade and caffeine can modulate neuroinflammation and afford neuroprotection to the retina against damage induced by I-R injury.

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IRB Status: None

Disclosures:

ANA RAQUEL SANTIAGO: No financial relationships to disclose

P309

CROSS-TALK BETWEEN AQUAPORINS AND INFLAMMATORY MEDIATORS IN GLAUCOMATOUS IRIS AND TRABECULAR MESHWORK

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NARAYANA NETHRALAYA EYE HOSPITAL, GLAUCOMA AND RESEARCH¹; NARAYANA NETHRALAYA EYE HOSPITAL, GENES REPAIR, REGENERATION IN OPHTHALMIC LABORATORY²; NARAYANA NETHRALAYA EYE HOSPITAL³

Aquaporins (AQPs), a group of membrane proteins, have been localized at a cellular level in the human eye (AQPs 0-5, 7, 9 and 11). As AQPs facilitate water and small solute transport across the plasma membrane, they have been hypothesized to have a role in the pathogenesis of glaucoma. The role of inflammatory mediators has been demonstrated in glaucoma as well. However, the role of AQPs and any possible correlation with inflammatory deregulation is unknown in glaucoma. We evaluated the gene expression levels of AQPs in patients with glaucoma and also determined the cross-talk with inflammatory mediators. Tissue samples of iris and trabecular meshwork (TM) were obtained during intraocular surgery from patients with glaucoma and compared to normals. We first tested AQP7, 9 and 11 for expression in iris and TM and observed that AQP11 was not expressed at all, while both AQP7 and 9 were expressed differentially between control and glaucoma samples in both tissues. The normalized transcript levels of AQP7 were 6.16 ± 1.6 in glaucoma iris compared to 2.8 ± 1.6 in controls. AQP9 transcript levels were also increased to 5.81 ± 1.7 in disease (3.58 ± 1.3 in controls). The cytokines, interleukin 6 (IL6) and interferon-beta (IFNβ), were both increased by 5-fold and 3-fold respectively in glaucoma patients' iris. The data support the hypothesis that glaucomatous tissues may be upregulating the AQPs as a stress mechanism to counter increased intraocular pressures. It has been reported that AQP expression is enhanced in the ciliary body, retinal ganglion cells and Müller cells in glaucoma while our data is the first report of the upregulation of AQP7 and 9 concurrently with pro-inflammatory cytokines in the iris. Inflammatory signaling is known to change the permeability of leukocytes, astrocytes and epithelia in disease, mechanistically involving deregulation of AQPs and the same may hold true for iris or TM in glaucoma.

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Disclosures:

RAJESH SASIKUMAR: No financial relationships to disclose

P310

CONSEQUENCE OF ELEVATED IOP IN MMP-9 DEFICIENT MICE ON BRN3A+ RGC COUNT

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Matrix metalloproteinases (MMPs) are known to be the mediators of extracellular matrix remodeling. Increased levels

of matrix metalloproteinases, particularly MMP-9, have been found in the aqueous humor of patients with glaucoma, however the exact role of MMP-9 in glaucomatous changes are not understood. Our previous results indicated that MMP-9 deficient mice exhibit elevated IOP, in the absence of any overt morphological changes in the anterior chamber (*Mol. Vis.*, 19, 684-95, 2013). In the current study, we investigated whether the elevated IOP in MMP-9KO mice leads to RGC death. Wild type and KO littermates at different age groups: 2-3 months, 3-4 months and a later time point of 6-8 months were studied. IOP was measured using the TonoLab rebound tonometer. To rule out that the elevated IOP is not due to a difference in central corneal thickness (CCT), we measured CCT between WT and KO mice using ultrasound pachymeter. The animals were sacrificed, eyes were enucleated and retinas (n=4) from both WT and KO animals were dissected. These retinas were then stained with Brn-3a antibody and cell count was performed using ImageJ. Our results demonstrate that IOP was significantly increased in MMP-9KO mice compared to control littermates at all ages examined. There was no difference in CCT demonstrating that the elevated IOP observed in MMP-9KO mice was not confounded by corneal thickness. Additionally, we found no observable difference in Brn3a+ RGC count between MMP9-WT and KO mice, suggesting that at these time points, the elevated IOP in MMP-9 KO mice did not lead to significant Brn3a+ RGC death. For further studies, we plan to examine earlier markers of retinal damage and gliosis in the retina as well as investigate how MMP-9 works in maintaining axonal integrity of the optic nerve.

IRB Status: Approved

Disclosures:

ANUJA SIWAKOTI: No financial relationships to disclose

P311

A GENOMIC LOCUS MODULATING GANGLION CELL DEATH FOLLOWING ELEVATED IOP IN THE MOUSE

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It appears that there is differential susceptibility of retinal ganglion cells (RGCs) to death following IOP elevation in human glaucoma. The present study defines a genomic locus modulating the RGC susceptibility to elevated IOP in the BXD RI (recombinant inbred) mouse strain. IOP was elevated by injecting magnetic microspheres into the anterior chamber and blocking the trabecular meshwork using a handheld magnet to impede drainage (Samsel et al, *IOVS* 2011; 52:1671-1675). The IOP was then measured over the next 21 days. For an animal to be included in the study the microsphere-injected eye must show an IOP elevation above 20 mmHg for two consecutive days or an IOP above 30 mmHg on a single day. On day 21, mice were sacrificed and the optic nerve was processed for microscopy. Axons were counted for both the injected and the control

eye. A total of 38 BXD strains (130 mice) were included in the analysis. The percentage axon loss for each strain was calculated and the data was entered into genenetwork.org. Using the Quantitative Trait Locus (QTL) mapping tool, we identified a significant QTL on Chromosome 18. Potential candidate genes in this peak were defined by determining genes with cisQTLs in the same locus. Seven candidates were identified (Adnp2, C16orf25, Pias2, Katnal2, Hdhd2, Skor2 and Smad2) and were evaluated for matches with human disease using the NEIGHBOR genome-wide association results. One gene (SMAD2) had a significant association ($p = 0.013$ when Bonferroni corrected for 7 candidates) with human normal tension glaucoma, but not with POAG overall. This approach identified a potential genetic risk factor for normal tension glaucoma (SMAD2). Interestingly, SMAD2 is part of the TGF β signaling pathway, which is associated with glaucoma.

IRB Status: Approved

Disclosures:

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P312

CUMULATIVE MTDNA DAMAGE AND MUTATIONS CONTRIBUTE TO THE PROGRESSIVE LOSS OF RGCs IN A RAT MODEL OF GLAUCOMA

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EYE AND ENT HOSPITAL OF FUDAN UNIVERSITY, RESEARCH CENTRE

Glaucoma is a chronic neurodegenerative disease characterized by the progressive loss of retinal ganglion cells (RGCs). Mitochondrial DNA (mtDNA) alterations have been documented as a key component of many neurodegenerative disorders. However, whether mtDNA alterations contribute to the progressive loss of RGCs and the mechanism whereby this could occur are poorly understood. We investigated mtDNA alterations in RGCs using a rat model of chronic intraocular hypertension and explored the mechanisms underlying progressive RGC loss. We demonstrate that the mtDNA damage and mutations triggered by intraocular pressure (IOP) elevation are initial and crucial events in a cascade leading to progressive RGC loss. Damage to and mutation of mtDNA, mitochondrial dysfunction, reduced levels of mtDNA repair/replication enzymes, and elevated reactive oxygen species form a positive feedback loop that produces irreversible mtDNA damage and mutation and contributes to progressive RGC loss, which occurs even after a return to normal IOP. Furthermore, we demonstrate that mtDNA damage and mutations increase the vulnerability of RGCs to elevated IOP and glutamate levels, which are among the most common glaucoma insults. This study suggests that therapeutic approaches that target mtDNA maintenance and repair and that promote energy production may prevent the progressive death of RGCs.

IRB Status: None

Disclosures:

JIHONG WU: No financial relationships to disclose

COMPARATIVE STUDIES OF THE ENDOTHELIAL GLYCOCALYX LAYER IN THE AQUEOUS OUTFLOW PATHWAY OF HUMAN AND BOVINE EYES

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The glycocalyx on the vascular endothelium plays an important role in mechanotransduction and permeability. We previously reported a non-uniform glycocalyx layer in bovine outflow pathway [JOVS 2013;54: E-Abstract 3033], but this layer remains uninvestigated in human outflow pathway. This study aims to compare the structure and distribution of glycocalyx in the bovine and human aqueous outflow pathways. Enucleated bovine (N=6) and human (N=2) eyes were either immerse- or perfuse- fixed with 1% glutaraldehyde and 4% paraformaldehyde in PBS and 0.05% Alcian Blue. Eyes were cut and immersed in 1% aqueous osmium tetroxide and 1% lanthanum nitrate followed by uranyl acetate and processed for electron microscopy. The glycocalyx distribution and thickness (in those regions where it was seen) were measured on the trabecular beams (TM), Schlemm's canal (SC)/aqueous plexus (AP), and collector channels (CC). The glycocalyx, which appears as a layer of hair-like brushes, was distributed non-uniformly in both bovine and human aqueous outflow pathways. However, the distribution was quite different in the two species. In bovine eyes, the fraction of the surface area covered with glycocalyx was found to be CC (37-75%) > TM (27-40%) > AP (5-28%), while in human eyes these fraction were found to be CC (59-81%) > SC (42-76%) > TM (15-34%). The glycocalyx was more uniform in the AP than TM in bovine eyes, whereas in human eyes, it was more uniform in SC than TM. Interestingly, in bovine eyes, the glycocalyx thickness was not significantly different at various regions (CC:80-113nm, AP:71-121nm, TM:62-127nm), whereas in human eyes, it was significantly thicker in CC (128-162nm) than SC (94-155nm), followed by TM (52-91nm). A non-uniform glycocalyx was found coating the endothelial surfaces in the bovine and human aqueous outflow pathways. The glycocalyx distribution and thickness appeared to be species-dependent. The implications of a discontinuous glycocalyx in the aqueous outflow pathway, unlike vascular endothelium, remain to be clarified.

IRB Status: Approved

Disclosures:

CHEN YUAN YANG: No financial relationships to disclose

A MICRORNA SIGNATURE OF RETINA IDENTIFIED BY DEEP SEQUENCING FOLLOWING CHRONIC OCULAR HYPERTENSION OF RAT

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EYE AND ENT HOSPITAL OF FUDAN UNIVERSITY

The molecular mechanism by which elevated intraocular pressure (IOP) induces glaucoma has come to be understood based on the identification of specific gene expression profiles. microRNA (miRNA) is a kind of short non-coding RNA which represses its target genes through mRNA degradation or protein translation blockage. Here we reported the miRNA signatures of IOP-induced glaucoma revealed by deep sequencing of rat retina. 941 known mature rat miRNAs and 213 novel rat miRNAs were identified. After clustering, it was found several clusters of miRNA were consistently responsive to increased pressure. Annotation of their targets by GO function and KEGG pathway turned out several well known glaucoma associated pathways like p53 and Wnt signaling pathways responded to IOP elevation and were involved in glaucoma development. Our findings reveal some potential miRNA markers for glaucoma and will provide insight into understanding of molecular mechanism of glaucoma progression.

IRB Status: None

Disclosures:

SHENGHAI ZHANG: No financial relationships to disclose

AN INVESTIGATION OF THE VARIATION IN VISUAL ACUITY THRESHOLDS MEASURED USING KAY PICTURE OPTOTYPES WHEN COMPARED TO A LANDOLT C TARGET

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In children, the accurate measurement of visual acuity (VA) is an important step in investigating visual function, particularly when amblyogenic factors are present. Significant differences in measured VA are found across a range of common pediatric charts; with the Kay Picture chart in particular producing significantly better VA for adults and children. Therefore we measured the legibility of individual Kay Picture optotypes to investigate whether this was due to differential visibility of specific optotypes, or a more global effect of optotype design. Adult participants (n=25) were assessed for best-corrected visual acuity (BCVA) using uncrowded Landolt C targets at 6m. Eight individual uncrowded Kay picture optotypes (1 arcmin stroke width at 3m, Snellen 3/3 equivalent) were presented 5 times in a random order at test distances of 3.8m-12m (in a logarithmic progression) until individual optotypes were correctly identified at less than the guessing rate (12.5%). The threshold distance for each optotype was found by fitting a 4th-order polynomial curve to the frequency of correct identification plot at each test distance. The threshold distance was interpolated from the fitted curves at the 56% correct response rate. Average threshold distances for each optotype were converted to logMAR VA for comparison. The mean threshold VA for the Landolt C was -0.19 ± 0.08 logMAR, while all Kay Picture optotypes produced better mean VA measurements ranging from -0.38 ± 0.13 logMAR for the Apple to -0.57 ± 0.10 logMAR for the Duck (ANOVA, $F=381.35$, $p<0.0001$). Further analysis showed that the mean VAs obtained for each optotype were all significantly different from one another. While Kay Pictures are constructed using Snellen

stroke width principles, we conclude that the optotypes produce higher measurements of VA due design elements such as overall shape, tilt and curvature of the pictures. These factors may allow hyperacuity to be utilized in the identification of the optotypes.

IRB Status: Approved

Disclosures:

ANDREW COLLINS: No financial relationships to disclose

P316

COMPLEMENT SYNTHESIS AND PROPAGATION BY IBA1+ MONOCYTES/MICROGLIA IN AMD AND IN ANIMAL MODELS OF AGING AND DEGENERATION

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Complement activation is associated with the pathogenesis of retinal dystrophies such as age-related macular degeneration (AMD). We aimed to examine the source of a crucial component of complement, C3, in the retina. These include animal models of retinal aging and light-induced degeneration, as well as human donor retinas with 'dry' and 'wet' forms of AMD. In the degenerative model, rats were exposed to 1000lux light for up to 24hrs, after which some animals were kept in dim light to recover. In the aging animal model, rats were reared in age-groups from post-natal (P) days 100, 450, and 750. For both models, animals were euthanized and retinas processed. Human donor tissue was collected and cryosectioned from patients with either 'wet' or 'dry' AMD. Expression of C3 was assessed by qPCR, immunohistochemistry, and in-situ hybridization, while immunoreactivity for IBA1 was utilized for monocytes/microglia. In the light-damage model, significant up-regulation of C3 by qPCR was observed during and following exposure, correlating with photoreceptor death. C3 expression also increased with age in rats, particularly at P750. In-situ hybridization, coupled with immunoreactivity for the monocyte marker IBA1, revealed that C3 is expressed by infiltrating monocytes/microglia associated with the inner retina and subretinal space following light-damage, and at P750 during aging. In AMD donor retinas, in-situ hybridization show that C3 is also expressed by IBA1+ monocytes/microglia situated in the inner retina, ONL and subretinal space, particularly in-and-around the lesion area. Our data demonstrate that IBA1+ monocytes contribute to prolonged activation of complement in both degenerative and aging retinal models though the local synthesis of C3. Crucially, we also show that these cells are responsible for synthesizing C3 in both 'wet' and 'dry' forms of AMD. Thus, the findings have relevance to the cellular events of complement activation underling the pathogenesis of AMD.

IRB Status: None

Disclosures:

MATTHEW RUTAR: No financial relationships to disclose

P317

REDUCTION OF ACUTE POST-OPERATIVE INFLAMMATION BY TOPICAL RX-10045 IN THE RABBIT PARACENTESIS MODEL

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RX-10045 is an analog of Resolvin E1, a potent anti-inflammatory mediator that is endogenously produced from omega-3 fatty acid. RX-10045 may be a safer alternative to corticosteroids and NSAIDs for control of ocular inflammation. This study evaluated the therapeutic effect of RX-10045 on post-operative ocular inflammation in a rabbit model.

RX-10045 (0.03% and 0.1%), dexamethasone HCL (0.1%; DEX), or placebo was applied topically to both eyes of NZW rabbits 180, 120, 90, and 30 minutes prior to, and 15, 30, and 90 minutes after initial paracentesis (IP) performed using a 27-gauge needle to aspirate 100 uL of aqueous humor (AH). Clinical microscopic ocular inflammatory scores (OIS) (Hackett-McDonald) and IOP (TonoVet) were recorded pre-dose then at 0.5, 1, 2, 4, and 6 hours after IP. Paracentesis was repeated, rabbits euthanized, and ocular tissues collected at 0.5, 2, or 6 hours after IP. Also evaluated were AH protein concentration (Bradford assay) and inflammatory cell infiltrate on ocular histopathology.

Mean cumulative OIS, aqueous flare (AF), and cellular flare (CF) in placebo treated eyes remained elevated through 6 hours after IP. Eyes treated with RX-10045 (0.03% only) and DEX had significantly lower mean cumulative OIS, AF, and CF than placebo at 0.5 hour (P<0.05), and RX-10045 (0.03% and 0.1%) and DEX had significantly lower mean cumulative OIS, AF, and CF at 1 and 2 hours (P<0.05) after IP. IOP between groups was similar before and after IP. AH protein and inflammatory cell infiltrate were lower in 0.03% RX-10045 and DEX treated eyes compared to placebo at 0.5, 2, and 6 hours after IP.

Therefore, topical 0.03% RX-10045 was as effective as DEX in reducing paracentesis-induced ocular inflammatory scores, AH protein, and cellular infiltrate. These results strongly support further investigation of RX-10045 as a novel, effective, and safe treatment for ocular inflammation.

IRB Status: None

Disclosures:

BRIAN GILGER: Consultant/Advisor relationship with Auvén Therapeutics

P318

IN VIVO IMAGING OF MICROGLIA IN CHOROIDAL NEOVASCULARIZATION

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Microglia coordinates pathological events in the retina. In order to assess microglia contribution in choroidal neovascular-

ization (CNV) events, we used *in vivo* imaging to study changes in the microglia population of the retina. *Csf1r*-EGFP mice provide a suitable model for studying macrophage-lineage cells due to their easy fluorescent visualization both *in vivo* and *in situ*. CNV was induced by laser lesions in triplicate following a straight line pattern and avoiding main vessels (Argon ion laser: 150 mV, 0.1 sec, 50 μ m). The microglia status was monitored at stages: D0, D1, D4, D7 and D14 using a laser scanner ophthalmoscope (LSO) to detect microglia fluorescence. As a standard procedure, at D14 eyes were enucleated and retinas collected for flat-mount preparation. *In situ* analysis of the vessels was assessed by isolectin B4 staining. *In situ*, we observed a defined population of microglia lying on the main large vessels of the retina. LSO allowed us to distinguish different microglia phenotypes: ramified (surveying), transitional (activated) and ameboid (activated). The follow-up analysis was performed by quantification of activated microglia separate at OPL and IPL, revealing OPL as the layer significantly holding the biggest amount of ameboid-like cells. We didn't detect significant levels of activated cells between 30-60 minutes directly after laser injury. However, the number of activated microglia rose after 24 hours, which level was maintained at least until 4 days after it decreased conserving high numbers by the time until 14 days after. We concluded that there is a subpopulation of microglia lying on vessels which is probably activated for angiogenesis. After laser injury there was no acute reaction, but it appeared after 24 hours and sustained over 14 days, starting prior to new blood vessel formation (D7). Thus, microglia suggests playing an active role in CNV formation.

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Disclosures:

SERGIO CRESPO-GARCIA: No financial relationships to disclose

P319

HIGH SPATIAL RESOLUTION IN VIVO MAGNETIC RESONANCE IMAGING OF THE HUMAN EYE, ORBIT, NERVUS OPTICUS AND OPTIC NERVE SHEATH AT 7.0 TESLA

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Imaging of the human eye *in vivo* at 7 Tesla is an advanced magnetic resonance imaging application and not yet a standard imaging modality for today's clinical routine. However, ultra high field magnetic resonance imaging of the human eye provides benefits for *in vivo* evaluation of anatomy and morphology in sub-millimeter spatial resolution for today's clinical science and for future clinical applications. To this end the purpose of the study was to examine the applicability of a 6 channel transceiver radiofrequency coil array in conjunction

with an optoacoustic triggering regime for imaging of the orbital and intracranial structures at 7 Tesla *in vivo*. Magnetic resonance imaging was performed with T1-weighted 3D fast low angle shot and 2D T2-weighted rapid acquisition with refocused echoes sequences. The six-channel coil array supports high spatial resolution imaging with an in plane resolution of 0.25 x 0.28 mm. This facilitates the depiction of anatomical details of the eye, the orbit, the optic nerve and the optical nerve sheath. Motion related artifacts could be eliminated using optoacoustic triggering regime. Our results underline the benefits of multi-element transceiver RF coil array technology and trigger protocols tailored for MRI eye applications *in vivo*.

IRB Status: International

Disclosures:

OLIVER STACHS: No financial relationships to disclose

P320

EFFECTS OF CYCLOHEXYLADENOSINE ON OUTFLOW FACILITY IN PERFUSED ENUCLEATED MOUSE EYES

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Adenosine can be delivered to trabecular meshwork (TM) adenosine receptors (ARs) *in vivo* by ectoenzymatic conversion of ATP released from TM cells. Selective activation of A1ARs lowers IOP in rabbits, monkeys and mice. A1AR-induced IOP reduction is mediated by increased outflow facility in monkeys and perfused bovine anterior segments, but the mechanism has not been identified in mice. Mice provide a favorable experimental model in being the lowest species on the evolutionary scale whose eyes display anatomical, physiological and pharmacological similarities to human eyes. Here, we test whether the selective A1AR agonist cyclohexyladenosine (CHA) lowers IOP by increasing conventional outflow facility in enucleated mouse eyes. Paired enucleated mouse eyes (C57BL/6) were perfused with media containing CHA (1 μ M or 10 μ M) or vehicle using a computer-controlled syringe pump to determine flow during four pressure steps (4, 8, 15 and 20 mmHg). Outflow facility was calculated from linear regression analysis of pressure-flow curves and compared. Perfusion with 1 μ M CHA did not significantly affect outflow facility (0.0263 ± 0.0066 μ L/min/mmHg, mean \pm SD) when compared to vehicle (0.0267 ± 0.0042 μ L/min/mmHg, mean \pm SD, $p=0.869$, $n=6$). However, perfusion with 10 μ M CHA significantly increased outflow facility (0.0379 ± 0.0234 μ L/min/mmHg, mean \pm SD) compared to vehicle (0.0216 ± 0.0110 μ L/min/mmHg, mean \pm SD, $p=0.042$, $n=6$). Our observed 75% increase in outflow facility was similar to the 71% increase reported in monkeys and significantly greater than the 28% increase in enucleated bovine eyes. Similar to bovine, we observed an effect of CHA on outflow facility within 1 hour. Our findings of robust adenosine A1 effects on conventional outflow facility indicate that the mouse eye is a particularly favorable model for elucidating the cascade of events from TM-cell ATP release to reduction in IOP.

IRB Status: Verified

Disclosures:

W. MICHAEL DISMUKE: No financial relationships to disclose

P321

SIGMA-1 RECEPTOR INCREASES MITOCHONDRIAL MEMBRANE POTENTIAL IN GLUCOSE AND OXYGEN DEPRIVED RETINAL GANGLION CELLS

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Understanding the role of mitochondria in retinal ganglion cells (RGCs) pathophysiology is relevant to human disease, as studies showed mitochondrial abnormalities in primary open angle glaucoma patients. Recent studies in our laboratory demonstrated that glucose and oxygen deprivation decreased mitochondrial movement in RGCs and sigma-1 receptor (σ -1r) agonists restored mitochondrial movement following glucose and oxygen deprivation. The current study determined the role of the σ -1r in regulating mitochondrial membrane potential in RGCs. RGCs were isolated from rat pups and subjected to glucose and oxygen deprivation in the presence or absence of σ -1r agonist and antagonist. In separate experiments, an adeno-associated viral vector encoding σ -1r (AAV- σ -1r) was used to increase σ -1r expression in primary RGCs and the cells were then subjected to oxygen/glucose deprivation. Mitochondrial membrane potential was measured using JC1 dye. Glucose and oxygen deprivation in RGCs resulted in decreased mitochondrial membrane potential when compared to normoxic RGCs. Addition of σ -1r agonists restored the mitochondrial membrane potential comparable to normoxic conditions while σ -1r antagonists abolished these effects. Overexpression of the σ -1r resulted in the restoration of mitochondrial membrane potential of RGCs following glucose and oxygen deprivation. These data suggest that σ -1r restores RGCs function following glucose and oxygen deprivation; particularly mitochondrial function which is vital to the health of the cells.

IRB Status: None

Disclosures:

DORETTE ELLIS: No financial relationships to disclose

P322

RETINAL WHITE BLOOD CELL FLUX AND SYSTEMIC BLOOD PRESSURE IN PATIENTS WITH TYPE 1 DIABETES

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Aims: There is evidence that altered retinal blood flow and altered retinal blood flow regulation play a role in the development and progression of diabetic retinopathy. We compared the association between systemic blood pressure and retinal white blood cell flux in patients with type 1 diabetes and healthy control subjects.

Methods: The study was performed in 100 patients with type 1 diabetes with no or minimal diabetic retinopathy and a group of 313 age-matched healthy controls. Inclusion criteria were systolic blood pressure ≤ 160 mmHg and diastolic blood pressure ≤ 95 mmHg. None of the subjects took vaso-active medication except insulin. The blue field entoptic technique was used to assess retinal white blood cell flux, velocity and density in the perimacular region. Pressure-flow relationships were calculated for both groups to assess differences in blood flow regulation.

Results: Retinal white blood cell flux was comparable between the two study groups. Both type 1 diabetic patients and healthy subjects showed a significant positive correlation between retinal white blood cell flux and mean arterial pressure (diabetic patients: $r = 0.48$; $p < 0.05$, healthy subjects $r = 0.28$). The correlation coefficients between mean arterial pressure and white blood cell flux were significantly higher in patients with diabetes than in the healthy control group ($p = 0.0459$).

Conclusion: Retinal white blood cell flux, as assessed with the blue-field entoptic technique, is not significantly different between type 1 diabetic patients with no or minimal retinopathy and healthy control subjects. Type 1 diabetic subjects do, however, show an abnormal association between systemic blood pressure and retinal white blood cell flux. This indicates altered autoregulation in early diabetic retinopathy.

IRB Status: International

Disclosures:

GABRIELE FUCHSJÄGER-MAYRL: No financial relationships to disclose

P323

CX3CR1 DEFICIENCY EXACERBATES PHOTORECEPTOR TOXICITY OF MONONUCLEAR PHAGOCYTE IN A MODEL OF SUBRETINAL INFLAMMATION

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UMRS 968

Mononuclear phagocytes (MP) accumulate in the vicinity of the atrophic lesion of the GA form of Age-related Macular Degeneration and are thought to contribute to photoreceptor degeneration. Similarly, CX3CR1^{-/-} mouse exhibit an age-dependent subretinal MP accumulation associated with an exacerbated photoreceptor cell death. The mechanisms by which monocytes that have entered the subretinal space trigger photoreceptor cell death is currently unknown. To analyze the regulation of monocyte derived MP neurotoxicity by subretinal environment and CX3CR1 deficiency, we isolated and co-cultured equal numbers of C57BL/6 and CX3CR1^{-/-} bone

marrow monocytes with C57BL/6 retinal explants or purified photoreceptor outer segment (POS). Control monocytes were cultured without overlying explant or POS. After 18 hours, MP mRNA expression of specific pro- or anti-inflammatory marker was assayed by qPCR. In specific experiments, TUNEL staining was performed on retinal explants to assay the neurotoxicity of MP C57BL/6 and CX3CR1^{-/-}. We here show that Cx3cr1 deficiency leads to an increase in photoreceptor toxicity of the ex-vivo differentiated MP. We further show that co-culturing Cx3cr1^{-/-} monocytes in the presence of an overlying retinal explants enhance the expression of inflammatory genes such as IL-1 β , TNF α and IL-6. In contrast, in similar conditions C57BL/6J monocytes express significantly higher levels of anti-inflammatory markers such as CD206, IL-1Ra. Our results demonstrate that ex-vivo co-culture of monocytes in contact with retinal explants or POS recapitulates in vivo MP differentiation and photoreceptor toxicity. This in vitro model will help at deciphering mechanisms involved in subretinal MP toxicity and test neurotoxic inhibitors that could prevent MP toxicity.

IRB Status: Approved

Disclosures:

SHULONG HU: No financial relationships to disclose

P324

RESTORING LIGHT SENSITIVITY IN BLIND RETINAE USING THIRD GENERATION PHOTOPHARMACOLOGY

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More than 300 million people worldwide are suffering from blindness or impaired vision. Although the photoreceptor cells in some cases undergo complete degeneration, the remains of the retinal circuitry are still functionally intact. In order to restore light sensitivity to blind retinæ, optochemical tools targeting receptors natively expressed in retinal cells provide a new photopharmacological strategy without the need of any genetic manipulation. We introduced the concept of photochromic ligands (PCL), which takes advantage of photoswitchable azobenzene molecules. These molecules are chemically modified in such a way that they enable optical control of neuronal activity by acting as selective photoswitchable receptor ligands or blockers of voltage-gated ion channels (VGIC). Together with the Kramer lab at UC Berkeley, we could show that photoswitchable VGIC blockers (Polosukhina et al. 2012, Tochitsky et al. 2014) are able to restore light sensitivity in genetically blind mice. However, the aforementioned molecules harbor difficulties in the application to the retina. We therefore developed Red-DAD, a third-generation PCL targeting VGICs. In multi-electrode array and whole-cell patch-clamp experiments we could demonstrate that Red-DAD is able to trigger retinal ganglion cell (RGC) spiking in a light-dependent fashion in blind mice. This molecule exhibits several features making it superior to previous PCLs for the application in vision restoration. First, the compound is not permanently charged and thereby crosses biological barriers more easily. Second, it is red-shifted, i.e. one can switch Red-DAD with white light. Third, lower light intensities in the range of bright ambient illumination are needed and finally, it relaxes back to

the *trans*-configuration within 200ms after turning of the light. Additionally, pharmacological approaches reveal that Red-DAD targets bipolar cells rather than retinal RGCs. By acting upstream of RGCs Red-DAD therefore has the advantage of providing a more complex signaling output, utilizing the intrinsic processing circuits of the retina.

IRB Status: None

Disclosures:

LAURA LAPRELL: No financial relationships to disclose

P325

MYOPIA PROGRESSION IN YOUNG ADULTS

YI PANG

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Purpose: To determine if there is myopia progression in young adult optometry students whose professional education requires extensive near work.

Methods: Medical records of 880 optometry students attending the Illinois College of Optometry were reviewed for the study. Enrollment criteria were: myopia at least -0.50 D, two eye exams during the optometric education which were at least 12 months apart, no ocular pathology, best corrected visual acuity 20/25 or better. A total of 405 subjects were qualified for the study. The following information was collected: date of exam, date of birth, gender, race, best corrected VA, phoric posture, and refraction from the binocular balance. Only the refraction in the right eye was used to data analysis. A paired student t-test was performed to compare the refractive error at the first to that at the second exams. One-way analysis of variance was performed to compare myopia progression in different races. Effects of gender and phoric posture on myopia progression were tested by student t-test.

Results: There were 274 female and 131 male in this study with the mean age of 23.60 (SD: 1.84) years. The mean change in myopia over an average period of 1.72-year was -0.18 D (SD: 0.40), with statistically significant difference detected between the refraction of the first exam (mean \pm SD: -3.96 \pm 2.35D) and that of the second exam (mean \pm SD: -4.15 \pm 2.39) ($P < 0.0001$). Myopia progression was -0.21 D in the subjects with exophoria compared to -0.17 D in those with esophoria without statistical significance. No difference in myopia progression was detected between male and female. Asian (-0.20 D) and Caucasian (-0.19 D) subjects had a trend of more myopia progression than African American (-0.03 D) subjects without statistical significance.

Conclusions: There was a statistically significant progression of myopia in these young adults during their optometric education; however, the myopia progression may be too small to be considered clinically significant.

IRB Status: Approved

Disclosures:

YI PANG: No financial relationships to disclose

NMDA AND AMPA RECEPTOR STIMULATION IN RETINAL GANGLION CELLS INDUCES PROLONGED PHOSPHORYLATION OF CREB AND INCREASES RESISTANCE TO APOPTOSIS

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NMDA and AMPA receptors are ionotropic glutamate receptors of the central nervous system (CNS) that have been shown to play dual role in mediating either neurodegeneration or neuroprotection through the CREB pathway. The purpose of this study was to investigate the role of NMDA and AMPA stimulation on the phosphorylation of CREB (pCREB) in a purified RGC culture. Additionally, we determined if overstimulation of these receptors in RGCs *in vitro*, can cause excitotoxicity as demonstrated in other neuronal cultures. Purification and the culture of RGCs were performed by an immunopanning technique using an antibody to Thy1.1 from P3-P7 Sprague-Dawley rats. RGCs were cultured for 7 DIV before s-AMPA (100 μ M) or NMDA (100 μ M) treatments. Calcium imaging measured cellular calcium influx and immunoblot analysis determined pCREB expression following NMDA or AMPA treatments. NMDA or s-AMPA mediated excitotoxicity in purified RGCs culture was determined by Caspase-3/7 luciferase activity assay, immunoblot analysis of cleavage of α -fodrin, and the Live (Calcein AM)/Dead (ethidium homodimer) assay. Treatment with either NMDA or s-AMPA for 6h significantly increased pCREB expression ($p < 0.05$) in purified RGC culture but not in the mixed retinal culture. MK801 (NMDA antagonist) blocked NMDA induction of pCREB in RGCs. Prolonged s-AMPA treatment (72h) did not alter caspase-3/7 activities and α -fodrin expression in RGCs. Both s-AMPA and NMDA treatments to RGCs did not induce cellular death in an oxygen/glucose deprivation model. However, in the mixed retinal culture, significant increase cellular death was observed following NMDA receptor stimulation. These results suggest that purified RGCs *in vitro* are not susceptible to AMPA or NMDA excitotoxicity as previously hypothesized. On the contrary, enhanced cell survival was observed following AMPA or NMDA treatments, possibly through prolonged CREB phosphorylation in purified RGCs. Furthermore, the present data suggests that stimulations of these receptors could be beneficial for RGC function and survival.

IRB Status: Approved

Disclosures:

YONG PARK: No financial relationships to disclose

PATIENT EXPERIENCE AND COMFORT DURING VITRECTOMY SURGERY WITH MONITORED ANESTHESIA CARE

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The purpose of the study was to quantify patient comfort and the visual and auditory experience during outpatient trans pars plana vitrectomy (TPPV) under monitored anesthesia care (MAC) with local anesthesia. We wished to determine what patients report regarding comfort with body positioning, betadine preparation, peribulbar anesthetic injection, and surgery on their first post-operative visit. A prospective observational study of 100 TPPV procedures performed by five surgeons was conducted at a single academic eye hospital on adults under MAC with peribulbar block between March and October 2013. The pre- and intraoperative intravenous and local anesthetics used were collected, and patients were given a postoperative questionnaire administered 1-2 days after surgery designed to elicit patient visual experience and comfort. Patients received a combination of midazolam, fentanyl and propofol for anesthesia. Twenty eight percent of patients reported seeing visual phenomenon (lights, colors), and 17% reported seeing moving instruments in the eye. Seventy percent of patients remember being in the operating room; 53% of patients remember body positioning and draping; 30% remembered receiving the "numbing injection"; and 29% remembered getting the eye prepped. Pain reported on scale of 1 to 10 (maximum pain) was 1.3 ± 0.6 for eye cleaning, 2.1 ± 1.8 for body positioning and drapes, 2.1 ± 1.6 for the numbing injection, and 2.9 ± 2.3 for the surgery. Sixty-six percent of patients reported hearing the surgical team talk, and 35% of patients remember talking intraoperatively. Eighty-eight percent of patients felt comfortable hearing talking during surgery. Though 26% of cases were over one hour long, only 5% of patients reported having preferred general anesthesia. In our prospective study designed to investigate patient comfort during the surgical experience, we found the prevalence of visual phenomenon to be lower than other groups. MAC with local anesthesia provided excellent analgesia and comfort for the vast majority of patients.

IRB Status: Approved

Disclosures:

HEMA RAMKUMAR: No financial relationships to disclose

THE EFFECT OF DOPAMINE D1 AND D2 AGONISTS AND ANTAGONISTS ON THE DEVELOPMENT OF FORM DEPRIVATION MYOPIA IN TREE SHREWS

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Eight groups received monocular form deprivation (FD) with a translucent diffuser, brief daily anesthesia (5% isoflurane), and 5 μ l intravitreal injections through a glass pipette inserted near the ora serrata for 11 days, starting at 24 days of visual experience. The untreated fellow eye served as a control. The NaCl group (n=6) received 0.85% NaCl vehicle. Two groups (n=4 each) received high (270 μ M) or low (27 μ M) doses of the

D1 agonist SKF38393 in vehicle. One group (n=4) received 620 μ M of the D1 antagonist SCH23390. Two groups received high and low doses (310 μ M, n=6; 31 μ M, n=3) of the D2 agonist quinpirole. Two groups received high and low doses (80 μ M, n=5; 31 μ M, n=3) of the D2 antagonist spiperone. Awake, non-cycloplegic refractions were measured daily. The NaCl group developed -4.7 ± 0.6 diopters (D) (mean \pm SEM) of myopia in the treated/injected eyes relative to the control eyes. The high dose of quinpirole significantly reduced the amount of myopia compared with the NaCl group (-2.0 ± 0.7 D; $p=0.018$). The high dose spiperone reduced the myopia compared with the NaCl (-1.7 ± 0.6 D; $p=0.009$) and low-dose spiperone (-4.8 ± 1.4 D; $p=0.041$) groups. The myopia in the two SKF38393 groups (high, -3.8 ± 1.0 ; low, -5.6 ± 1.3 D) and the SCH23390 group (-3.0 ± 0.8 D) did not differ significantly from the baseline group. The high doses used appeared to be slightly higher than the effective doses in chick. In the lower dose groups, some animals had reduced myopia while others did not. The results suggest a role for the dopamine D2 pathway in the regulation of refractive development in this mammal closely related to primates. The effectiveness of both D2 agonists and antagonists suggests that a balance of dopamine activity is important.

IRB Status: None

Disclosures:

ALEX WARD: No financial relationships to disclose

P329

MODULATION OF PRO-INFLAMMATORY RESPONSES IN THE RETINA BY NEUROPEPTIDE Y: THE ROLE OF NPY Y1 RECEPTOR

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Neuropeptide Y (NPY) is a neuromodulator in central nervous system and a regulator of inflammatory processes. Sitagliptin is an antidiabetic drug that inhibits dipeptidyl-peptidase-IV, an enzyme that processes NPY. Since neuroinflammation and microglia activation have a predominant role in the progression of retinal degenerative diseases, we investigated whether NPY, and particularly NPY Y1 receptor (Y1R) activation, and sitagliptin, could inhibit neuroinflammation in the retina. Retinal microglial cells were immunoreactive to NPY and NPY Y1, Y2 and Y5 receptors. NPY and Y1R activation prevented LPS-induced microglia morphological changes in retinal explants. Also, 1 μ M NPY or 1 μ M [Leu31, Pro34]-NPY (LP-NPY; Y1R/Y5R agonist) inhibited the increase in iNOS immunoreactivity and mRNA expression triggered by 3 μ g/mL LPS. BIBP3226 (Y1R antagonist; 1 μ M) abolished the effect of LP-NPY. LP-NPY also inhibited the LPS-induced increase in TNF and IL-1 β , and BIBP3226 abrogated the effect of LP-NPY. In a retinal ischemia-reperfusion animal model, intravitreal injection of 10 μ g NPY before ischemia inhibited morphological changes in retinal microglia, assessed 24h after reperfusion. Furthermore, 8h after reperfusion the up-

regulation of TNF, IL-1 β and IL-6 mRNA, and the production of TNF and IL-6, in ischemic retinas, were inhibited by NPY. Sitagliptin also prevented LPS-induced microglia morphological changes in retinal explants and inhibited the increase in iNOS immunoreactivity driven by LPS in primary retinal mixed cultures. Preliminary results suggest that Y1R blockade is able to decrease, at least partially, the inhibitory effects of sitagliptin on inflammation. This study shows that the modulation of NPY system, particularly Y1R activation, is able to control neuroinflammation in the retina, suggesting NPY system as a potential therapeutic target for retinal diseases with an inflammatory component. Sitagliptin might also be seen as an anti-inflammatory drug able to inhibit neuroinflammation in the retina.

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P330

A ROLE OF THE HOMEOPROTEIN TRANSCRIPTION FACTOR RAX IN POSTNATAL PHOTORECEPTOR DEVELOPMENT

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The homeoprotein transcription factor *Rax* is an essential regulator of eye formation. *Rax*-deficient mouse embryos show a complete loss of the optic vesicle. However, the molecular function of *Rax* in late retinal development remains largely unknown. We previously reported that *Rax* activates the expression of transcription factor *Otx2* in the last cell cycle of retinal progenitors and promotes photoreceptor cell fate in the embryonic retina (Muranishi *et al.*, 2011, *J. Neurosci.* 31; 16792-16807). In the present study, we examined *Rax* function at postnatal stages. We generated an inducible *Rax* conditional knockout (*Rax* iCKO) mouse line in which *Rax* can be deleted in photoreceptor cells upon tamoxifen administration. In *Rax* iCKO mouse retinas, the expression of some of photoreceptor genes was significantly reduced. To identify genes regulated by *Rax*, we performed genome-wide microarray analysis. These results demonstrate that *Rax* controls the expression of cone and rod photoreceptor genes in the postnatal retina. Our study reveals a functional role of *Rax* *in vivo* in postnatal photoreceptor development.

IRB Status: None

Disclosures:

SHOICHI IRIE: No financial relationships to disclose

SELECTIVE ABLATION OF VASCULATURE AFFECTS RETINAL NEUROGENESIS IN ZEBRAFISH EMBRYOS

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The role of vascular endothelial cells, especially those of the hyaloid vasculature, in regulating the development and neurogenesis of the retina remains poorly understood. Here, we selectively ablated endothelial cells in developing zebrafish embryos by crossing two transgenic lines: *Tg(cdh5:gal4)* and *Tg(UAS-E1b:Eco.NfsB-mCherry)*. In the doubly transgenic embryos, Gal4, a yeast transcription factor, binds with the enhancer UAS to express m-Cherry and *E. coli* nitroreductase in the cells expressing cadherin5 (endothelial cells). The doubly transgenic embryos were then treated with a prodrug Metronidazole (Mtz) so that nitroreductase converted Mtz into a toxic compound that crosslinks with the DNA, leading to vascular endothelial cell death. As an alternative strategy for eliminating vascular cells, we treated wildtype zebrafish embryos with the VEGF receptor signaling inhibitor, SU5416, which leads to the absence of blood vessels. The embryos were evaluated for eye and lens size, retinal lamination, photoreceptor differentiation and other retinal neuronal type differentiation using histological and microscopic techniques. Our results indicate that the absence of vasculature leads to microphthalmic eyes, disrupted retinal lamination and defects in retinal neuronal differentiation. Our results also indicate that the observed phenotypes are not the consequences of hypoxia, further suggesting that vasculature is required for more than metabolic purposes during retinal neurogenesis. Therefore, our results suggest that a regulatory relationship exists between the vasculature and the process of retinal neurogenesis, which could be endocrine or paracrine in nature. To distinguish between these alternatives, we evaluated the retinal phenotypes in the silent heart mutants which have normal vasculature but lack a contractile heart due to mutation in *Troponin2a* gene and hence have no circulation in their body. Initial neurogenesis in the silent heart mutants is not affected suggesting that vasculature has a paracrine function in regulating retinal neurogenesis.

IRB Status: None

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CHARACTERIZATION OF A BLIMP 1-SPECIFIC ENHANCER IN THE DEVELOPING RETINA

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The mechanisms that control the earliest events in photoreceptor development are incompletely understood. The transcription factor Otx2 is necessary for both photoreceptor

and bipolar interneuron formation. The transcription factor Blimp1 (Prdm1) is expressed in nascent Otx2+ cells, where it promotes photoreceptor formation by blocking bipolar fate. To identify factors that work with Otx2 to specifically promote photoreceptor genesis, we investigated the cis-regulatory network that controls Blimp1 expression. Using DNase hypersensitivity sequencing we identified 10 potential enhancer elements within 200kb of *Blimp1*. These elements were cloned into reporter plasmids containing a minimal promoter driving nuclear-localized GFP. Explanted newborn mouse retinas were co-electroporated with the enhancer constructs and a plasmid that ubiquitously expressed nuclear-localized Cherry. After one day of culture, explants were screened by immunohistochemistry to determine what fraction of GFP+ and Cherry+ cells co-expressed Blimp1. Only 1 region (1.9kb) recapitulated Blimp1 expression in retinal explant cultures. This region was subdivided into 3 fragments based on evolutionary conservation. The only fragment that recapitulated Blimp1 expression contained 2 highly conserved Otx2 binding sites. Mutagenesis of one of these sites resulted in partial loss of enhancer activity, while mutagenesis of the other led to complete loss of activity. This showed that Otx2 was necessary for activation of the Blimp1 enhancer. To test whether Otx2 is sufficient for enhancer activation, we tested a 189bp fragment that contained both Otx2 binding sites and the intervening sequence. This construct did not recapitulate Blimp1 expression, demonstrating that Otx2 binding is not sufficient for enhancer activation. These data suggest that combinatorial action of Otx2 and an as of yet unidentified factor are necessary for Blimp1 expression and photoreceptor formation during retinal development. Future experiments will characterize this element in vivo and assess its regulation by other transcription factors.

IRB Status: Verified

Disclosures:

JOSEPH BRZEZINSKI: No financial relationships to disclose

HYPERGLYCEMIA-DEPENDENT AND -INDEPENDENT ACTIVATION OF CASPASE-1 IN MÜLLER CELLS

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Increased caspase-1 activity and subsequent IL-1 (interleukin-1) production is a prominent feature of diabetic retinopathy. Type 1 IL-1 receptor knockdown (IL-1R1^{-/-}) mice are protected from disease development. Nothing is known about mechanisms leading to activation of the caspase-1/IL-1 pathway in high glucose conditions or how IL-1R1^{-/-} mice are protected from disease development. This study shows that caspase-1 activity is significantly increased in diabetic wild-type mice at 10 and 20 weeks. In IL-1R1^{-/-} mice however, caspase-1 activity is increased at 10 weeks of diabetes but not at 20 weeks. *In vitro* in primary human Müller cells (hMCs) caspase-1 activity is significantly increased after 48 and 96 hours of high glucose treatment but pretreatment with IL-1 receptor antagonist (IL-1ra) inhibited high-glucose induced caspase-1 activity at 96 hours. This indicates that caspase-1 activation can be separated into two

phases, the first phase dependent on high glucose alone and the second phase dependent on IL-1 and functional IL-1R1 signaling. Treatment with exogenous IL-1 β significantly increased caspase-1 activity independently of high glucose, confirming that IL-1 via IL-1R1 signaling can induce caspase-1 activity. To determine how caspase-1 is activated in high glucose conditions, Receptor Interacting Protein-2 (RIP2), a known activator of caspase-1, was examined. In hMCs, RIP2 was significantly upregulated by both high glucose and exogenous IL-1 β . siRNA-mediated knockdown of RIP2 significantly decreased caspase-1 activity induced by high glucose and IL-1 β . These studies suggest that RIP2 is a regulator of caspase-1 in both phases. Finally, sustained caspase-1 activation lead to Müller cell death both *in vivo* and *in vitro*, and inhibition of IL-1R1 signaling *in vivo* using IL-1R1 $^{-/-}$ mice or *in vitro* by IL-1ra in hMCs significantly decreased cell death. These studies suggest that interfering in the RIP2/caspase-1/IL-1/IL-1R1 pathway might present a novel therapeutic strategy to treat diabetic retinopathy.

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P334

FUNCTIONAL ROLES OF RAX HOMEOPROTEIN IN MOUSE RETINA DEVELOPMENT

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Establishment of the vertebrate retina requires a series of developmental steps including specification of the anterior neural plate, evagination of the optic vesicles from the ventral forebrain, and differentiation of cells. We previously identified a *paired*-type homeoprotein Rax (retina and anterior neural fold homeobox), which is highly expressed in mouse retinal progenitors. Rax is evolutionarily well conserved from *Drosophila* to human. Rax begins to be expressed in the anterior neural region of developing mouse embryos, and later in the retina, pituitary gland, hypothalamus, and pineal gland. Rax has an essential role of eye and forebrain development of vertebrate species. In humans, mutations in the RAX gene lead to anophthalmia and microphthalmia. Rax-deficient mouse embryos show a loss of the optic vesicle and abnormal forebrain formation. In addition, Rax regulates photoreceptor cell fate determination. We recently identified a *cis*-regulatory region which is responsible for *Otx2* expression in photoreceptor precursors, and we named this enhancer region "EELPOT" (embryonic enhancer locus for photoreceptor *Otx2* transcription). We showed that Rax homeoprotein interacts with EELPOT to activate the expression of transcription factor *Otx2* in the last cell cycle of retinal progenitors and induces photoreceptor cell fate in the embryonic retina. In the current study we further examined Rax function at postnatal stages. We generated an inducible Rax conditional knockout (Rax iCKO) mouse line, and performed induced deletion of Rax in photoreceptors at

postnatal stages. Our study reveals the essential function of Rax in postnatal photoreceptor development as well as that in embryonic development.

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TAKAHISA FURUKAWA: No financial relationships to disclose

P335

CONTRIBUTIONS OF VEGF AND PHOSPHORYLATION OF NITRIC OXIDE SYNTHASE IN THE DEVELOPMENT OF RETINOPATHY OF PREMATURITY

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Retinopathy of prematurity (ROP), contributes to 50,000 annual cases of neonatal blindness worldwide. We hypothesized that retinal hypoxia caused by regional retinal avascularity in ROP promotes phosphorylation and activation of endothelial nitric oxide synthase (eNOS), precipitating local vasodilatory changes that promote retinal angiogenesis. The effect of oscillating oxygen exposure on eNOS phosphorylation at Ser1179 (PeNOS) and vascular endothelial growth factor (VEGF) expression associated with ROP stage-progression was evaluated. Sprague-Dawley neonates were placed in an oxygen chamber, cycling at 50%/10% FiO₂/every 24 hours, for 14 post-natal days (P1-P14). On P15 pups were exposed to ambient 21% oxygen. Littermate non-treated naïve pups served as controls. Rats were euthanized on P15, P18 & P20 (n=22/group). One retina of each rat was flat-mounted and ADPase stained for vasculature evaluation. Homogenized contralateral retinas were evaluated using RT PCR for VEGF and eNOS mRNA levels. The PeNOS to total eNOS protein ratio was determined by relative band densities on western blots. Retinal cross-sections from a cohort treatment group were immunohistochemically stained for localization of VEGF, eNOS and PeNOS. Avascular retinal area in the treated group differed significantly from the fully-vascularized controls. The avascular area in the treatment group was largest at P15 with a decreasing trend by P18 and P20 (p>0.05). VEGF and eNOS mRNA levels on P18 and P20 as well as the PeNOS to total eNOS protein ratio at P20 were significantly higher than controls and P15. Greater immunoreactivity of VEGF, eNOS and PeNOS protein was detected by immunohistochemistry staining in various retinal cell layers, compared to controls. In summary, an increase in VEGF, eNOS, and PeNOS mRNA and protein expression correlate with increases in area of vascularity. These findings suggest that PeNOS and VEGF are associated with proangiogenic mechanisms in the ROP rat model.

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UNRAVELING THE THERAPEUTIC MECHANISMS OF HUMAN UMBILICAL CORD TISSUE – DERIVED CELLS (HUTC) IN RETINAL DEGENERATIVE DISEASES

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Age-related macular degeneration (AMD) is the leading cause of vision loss in people over the age of 50. Subretinal administration of hUTC to a model of retinal degeneration preserved photoreceptors and visual function; however, the therapeutic mechanism of hUTC has not been defined. Here we characterized the effects of hUTC on functional synapse formation, neuronal survival and outgrowth. Retinal ganglion cells (RGCs), purified from P7 rats, were co-cultured with hUTC or cortical astrocytes (ASCs) as a positive control. A normal human dermal fibroblast (NHDF) cell line was used as negative control. Synapse formation was assessed by immunocytochemical co-localization of pre- (Bassoon) and post-synaptic (Homer) markers and we found that RGC exhibited significant increases on both number and size of synaptic puncta, comparable to astrocytes (positive control). Subsequent electrophysiological recording of the RGCs treated under same conditions revealed that hUTC also increase the amplitude of miniature excitatory postsynaptic currents (mEPSCs). To confirm the effects of co-culture, RGCs were fed with various concentrations of hUTC-conditioned medium (hUCM). Synapse analysis showed that hUCM is sufficient to induce synapse formation in concentration dependent manner, similar to astrocyte-conditioned media (ACM). hUCM also strengthened functional synapses as shown by increased amplitude and frequency of mEPSCs. Besides its effects on synapse formation, hUCM was sufficient to promote RGC survival in the absence of any other growth factors. hUCM also enhanced RGC neurite outgrowth as demonstrated by significant increase in total process length, number of processes and number of branches. In conclusion, we found that hUTC secrete factors that promote development of functional synapses between purified RGCs in vitro. Moreover, hUTC also support neuronal survival and growth. Our findings suggest that hUTC may affect multiple aspects of retinal cell health and connectivity.

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THE ROLE OF THE ACTIN CYTOSKELETON AND RHO-ASSOCIATED COILED-COIL KINASES IN THE ADULT REGENERATING ZEBRAFISH RETINA

MANUELA LAHNE, David Hyde

In the adult zebrafish retina, dying photoreceptors induce Müller glia to divide and produce neuronal progenitor cells (NPCs) that subsequently differentiate and replace lost photoreceptors. Similar to NPCs undergoing interkinetic nuclear migration (INM) during retinal development, proliferating Müller glia nuclei migrate between the basal and apical limits of the retina in phase with the cell cycle during regeneration. Actin-myosin mediated contraction facilitates INM during retinal development. To test whether a similar mechanism acts during retinal regeneration, light-damaged Tg[*gfap*:EGFP] transgenic zebrafish that express EGFP in Müller glia, were exposed to CytochalasinD or Rockout, an actin polymerization or Rho-associated coiled-coil kinase (Rock) inhibitor, respectively. Immunocytochemical labeling of the mitosis marker phospho-histone 3 (pH3) was used to assess the position of dividing Müller glia nuclei in drug exposed retinal sections. At 35 hours of light treatment, pH3-positive Müller glial nuclei in DMSO controls had predominantly migrated apically within the inner nuclear layer (INL) or into the outer nuclear layer (ONL). In contrast, both CytochalasinD and Rockout caused significant increases in the number of pH3-positive Müller glial nuclei in the basal and apical INL at the expense of those in the ONL. Disrupting INM by inhibiting Rock significantly reduced the number of proliferating cells at 45 and 72 hours of light treatment. EdU and BrdU pulse-chase experiments which labeled activated Müller glia and dividing NPCs, respectively, revealed fewer EdU- and BrdU-double-positive cells at 45 and 50 hours of light treatment in Rockout-exposed retinas, suggesting premature cell cycle exit. Moreover, significantly fewer blue and red cones were regenerated at 8 days of recovery (post light-damage) in Rock-inhibited retinas relative to DMSO controls. In conclusion, disrupting the actin cytoskeleton interferes with INM, NPC proliferation and photoreceptor regeneration.

IRB Status: Approved

Disclosures:

MANUELA LAHNE: No financial relationships to disclose

HYPERGLYCEMIA INDUCES PYROPTOTIC CELL DEATH IN RETINAL MÜLLER CELLS

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Retinal cell death is a prominent feature in the progression of diabetic retinopathy. Most emphasis has been directed towards identifying apoptosis in the diabetic retina. However, new research has established that there are multiple types of cell death, with many more subtypes, besides apoptosis. An emerging type of cell death is 'pyroptosis.' Pyroptosis is an inherently inflammatory-mediated form of cell death, defined as being strictly dependent on caspase-1 activation. Previous work by us has demonstrated that diabetes leads to activation of caspase-1 in retinas of diabetic and galactosemic mice as well as tissue of diabetic patients. Inhibition of

the caspase-1 pathway prevented diabetic retinopathy in animal models indicating that this inflammatory pathway is important for disease development. Müller cells are a prominent source of active caspase-1 in the diabetic retina. Therefore, the goal of this study was to determine whether hyperglycemia leads to pyroptosis in Müller cells. Results demonstrate that hyperglycemia leads to significant cell death of Müller cell death *in vitro* and *in vivo* that depends on the activation of caspase-1, the most prominent indicator of pyroptotic cell death. Müller cell death is further promoted by caspase-1-dependent caspase-6 and caspase-3-like activation. Interestingly, despite significant increase in caspase-3-like activity, this activation did not lead to iCAD (inhibitor-of-caspase-activated DNase) activation and PARP (poly-ADP-ribose-polymerase) cleavage commonly associated with caspase-3 activation during apoptosis. In addition, hyperglycemia-induced cell death was not detectable by TUNEL staining but by trypan blue exclusion assay strongly indicating that hyperglycemia does not induce apoptotic cell death but rather pyroptosis in Müller cells. A better understanding of mechanisms leading to cell death by hyperglycemia will help to identify new therapeutic strategies to prevent cell death and potentially the progression of diabetic retinopathy in the future.

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SUSANNE MOHR: No financial relationships to disclose

P339

REGENERATIVE EFFECT OF TAURINE-CONJUGATED URSODEOXYCHOLIC ACID AND NEUROTROPHIN-4 IN RAT RETINAS EXPOSED TO HIGH GLUCOSE

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The purpose of this study is to examine the regenerative effect of Taurine-conjugated ursodeoxycholic acid (TUDCA) and neurotrophin-4 (NT-4) in cultured rat retinas exposed to high-glucose (HG). Retinal explants were cultured in normal-glucose (NG) and HG medium. After 7 days, the numbers of regenerating neurites were counted per explant. In the HG group supplemented with TUDCA (HG+TUDCA) and NT-4 (HG+NT-4), the numbers of neurites were significantly higher than in the HG group without TUDCA and NT-4. The number of neurites in HG+NT-4 was significantly higher than that of HG+TUDCA. Our previous study indicates that the survival effect of TUDCA was similar to that of NT-4 (Oshitari et al. Neurosci Lett 2011). Taken together, these results indicate that not only anti-endoplasmic reticulum stress-related mechanisms but also other molecular mechanisms were involved in the regenerative effect of NT-4 in retinas exposed to HG.

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Disclosures:

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P340

HIGH GLUCOSE ALTERS RETINAL ASTROCYTES PHENOTYPE THROUGH INCREASED PRODUCTION OF INFLAMMATORY CYTOKINES AND OXIDATIVE STRESS

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Astrocytes are macroglial cells with a crucial role in the development of retinal vasculature and maintenance of blood-retina-barrier (BRB). Diabetes affects the physiology and function of retinal vascular cells including astrocytes (AC) leading to breakdown of BRB. However, the detailed cellular mechanisms leading to dysfunction of retinal AC under high glucose conditions remain unclear. Here we show that high glucose conditions did not induce the apoptosis of retinal AC, but instead increased their proliferation and adhesion to extracellular matrix proteins. These alterations were associated with changes in intracellular signaling pathways involved in cell survival, migration and proliferation. High glucose conditions also affected the expression of inflammatory cytokines in retinal AC. In addition, we showed that the attenuation of retinal AC migration under high glucose conditions and capillary morphogenesis of retinal endothelial cells on Matrigel were mediated through increased oxidative stress and high glucose conditions preventing network organization of retinal AC on Matrigel. Antioxidant proteins including heme oxygenase-1 and peroxiredoxin-2 levels were also increased in retinal AC under high glucose conditions through nuclear localization of transcription factor nuclear factor-erythroid 2-related factor-2. Together our results demonstrated that high glucose conditions alter the function of retinal AC by increased production of inflammatory cytokines and oxidative stress with significant impact on their proliferation, adhesion, and migration.

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NADER SHEIBANI: No financial relationships to disclose

P341

GANGLION CELL LOSS IN RETINAL ISCHEMIA IS FACILITATED BY THE INFLAMMASOME

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Retinal ganglion cell (RGC) loss is characteristic to glaucoma, multiple sclerosis-induced optic neuritis, retinal ischemia and other pathologies. The neurotoxicity in these diseases is commonly associated with activation of pro-inflammatory pathways in the retina, particularly the NALP inflammasome complex that controls production of the IL-1 β /IL-18 cytokines. In this study, we tested a hypothesis stating that pathological induction of NALP

inflammasome is essential for RGC death in ischemia-reperfusion. We utilized a model of unilateral retinal ischemia-reperfusion (IR) and pannexin1 (Panx1), and caspase-1 (Casp1) knockout mouse lines with impaired inflammasome activation. Probenecid treatment was used for pharmacological blockade of Panx1 channels. Our data show that Panx1 and Casp1 deficiency resulted in a 97% and 98% RGC survival, respectively, a significant increase relative to that in wild type (WT) retinas. The same degree of protection was observed in mice treated with Panx1 inhibitor probenecid. Neuro2A cells expressing high levels of Panx1 also showed increased sensitivity (only 60% survived) to oxygen-glucose deprivation (OGD) when compared to control cells lacking Panx1 (97%) or cells treated with probenecid. Ischemia-induced levels of inflammasome components caspase-1 and caspase-11, as well as mature IL-1 β observed in control retinas or Neuro2A cells, were significantly decreased in the retinas of Panx1-null and WT probenecid-treated mice, as well as Panx1-null or probenecid-treated Neuro2A cells exposed to ischemia. Our results indicate that Panx1-mediated activation of inflammasome render neurons, particularly RGCs highly vulnerable to ischemic injury. Targeting inflammasome represent a feasible neuroprotective strategy in retinal pathologies with significant ischemic and inflammatory components.

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P342

DISRUPTION OF NEURON LAMINATION IN THE MOUSE RETINA ALTERS DEVELOPMENT OF THE RETINAL VASCULATURE

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Retinal vasculature development is a complex process involving coordination of environmental cues and cell signaling to establish a closed vasculature network capable of supplying tissues with the necessary oxygen and nutrients required to carry out their function. Much of the previous work on the developing retinal vasculature focuses on the development of the superficial plexus, yet development of the intermediate and deep plexus have been less explored. The present study used *Bax*^{-/-}, *BBC3*^{-/-} and *Dscam*^{-/-} mice to investigate the role of cell number and neuron lamination on the development of the retinal vasculature. *Bax* and *BBC3* are proapoptotic factors and the *Bax*^{-/-} and *BBC3*^{-/-} retinas have increased cell numbers. A similar increase in cell number is observed in the *Dscam*^{-/-} retina, along with disorganization of retinal lamination and mosaic patterning of neural soma. To determine if cell number and/or organization is required for normal blood vessel projection and lamination, the vessels in *Dscam*^{-/-}, *Bax*^{-/-} and *BBC3*^{-/-} retinas were compared to controls. *Bax*^{-/-} and *BBC3*^{-/-} retinas contain significantly more cells within the RGL and INL compared to WT

controls. *Dscam*^{-/-} retinas contain significantly more cells within all 3 nuclear layers compared to WT controls. No significant difference in cell number was observed between *Bax*^{-/-}, *BBC3*^{-/-}, and *Dscam*^{-/-} retinas. *Bax*^{-/-}, *BBC3*^{-/-}, and *Dscam*^{-/-} retinas have an increased amount of vessels. *Bax*^{-/-} and *BBC3*^{-/-} retinas plexus formation and diving of blood vessels resemble the wild type control. *Dscam*^{-/-} retinas develop a superficial and deep plexus, but lack a developed intermediate plexus. Vessels within the IPL of *Dscam*^{-/-} retinas take tortuous trajectories when diving into the deeper portions of the retina. As late as p35, filopodia have been observed in *Dscam*^{-/-} retinas within the inner plexiform layer. These data taken together suggest that proper neuron lamination is important for vessel plexus formation.

IRB Status: Approved

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P343

VISUAL PROPERTIES OF RCS RATS TRANSDUCED WITH MODIFIED VOLVOX CHANNELRHODOPSIN-1

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Volvox-derived channelrhodopsin, VChR1, has a broader, red-shifted action spectrum that is useful for restoring vision. However the ion channel properties is relatively low comparing with that of channelrhodopsin-2 because of low plasma membrane integration. We generated modified Volvox channelrhodopsin-1 (mVChR1), which is a chimera of *Chlamydomonas* channelrhodopsin-1 and Volvox channelrhodopsin-1 and demonstrated increased plasma membrane integration and dramatic improvement in its channel properties. To investigate the visual properties of mVChR1-transferred RCS rats, behavioral assessments using an optomotor were performed at least 2 months of the AAV-mVChR1 injection. A virtual optomotor which had the maximum light intensity up to 800 lux was newly developed for this study. Spatial frequencies, color and the speed of the rotation were controlled by a software. We displayed various spatial frequencies of blue-, green-, yellow-, and red-black stripes in the front of the rat with the rotation speed at 12 degrees per second. The rat tracked the stimulus displaying blue-, green-, yellow-, or red-black stripes by turning its head. However there are some differences of responsiveness to the color of stripes. Most of rats tracked the stimulus of 0.42 cycle per degree (CPD). In the case of the stimulus of green-black stripes, the rats could not respond to 0.42 CPD. The visual properties obtained by the behavioral studies were reflected in the results of patch clamp recording using mVChR1-transduced HEK 293 cells that the light sensitivity of mVChR1 at 500 nm was lower than those of other wavelengths. These results suggest that mVChR1 gene therapy may restore the entire visual spectrum for patients with RP through the transfer of only 1 gene.

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DSCAM MEDIATES DENDRITE LAMINATION PATTERN IN THE MOUSE RETINA IN A DOSE DEPENDENT MANNER BY RESTRICTING THE STRATUM IN WHICH NEURITES ARBORIZE

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Neural organization of the inner plexiform layer (IPL) is mediated by a combination of cell adhesion molecules that guide the dendrites and axons of cells into appropriate lamination, although much remains unknown concerning the mechanisms that control dendritic refinement in the retina. The IPL of the retina is organized into five stratum, S1-S5. By taking advantage of a transgenic mouse strain that expresses GFP in a population of amacrine cells early in development, we were able to assay early stratification of the population. We tracked the development of these cells' dendrites from postnatal day 1 through adulthood to determine the mechanism behind their arborization pattern that normally targets the S3 stratum. In wild type, it appears that this population of amacrine cell forms multidirectional dendrites in early development, but then refine to a consistent pattern of lamination by adulthood with dendrites exclusively to the S3 layer. Next we assayed how this arborization pattern is regulated. We find that the Down syndrome cell adhesion molecule (*Dscam*) gene is required for a number of aspects of this cell types' organization. Loss of function *Dscam* mutant mice show increases in overall cell number and ectopic dendritic adhesion compared to wild type, consistent with other cell types that have been studied in the *Dscam* and *Dscam11* loss of function retina. DSCAM protein was shown to restrict arborization of these cells' dendrites to S3. A dosage dependent reorganization of these cells' dendrites to targets S3 and S1 was observed. We assayed development of these neurites to determine if they represent ectopic dendrite growth or a refinement failure. Data suggests that DSCAM plays a dose dependent role in the normal development of this amacrine cell arborization exclusively into the S3 layer, and a loss of function results in a bi-stratified lamination with the S1 layer.

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P345

ANALYSIS OF ISOLATED ROD PHOTORECEPTORS FROM TRANSGENIC ZEBRAFISH

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Many human retinal degenerative disorders involve the death of rod photoreceptor cells. In these disorders, rods cannot be replaced, and their absence results in the subsequent death of cone photoreceptors, and blindness. The purpose of the current study is to identify genetic transcripts that are enriched in rod photoreceptors, in order to gain insights into intrinsic mechanisms underlying rod development and survival. We use the zebrafish as a model organism for this study, due to structural and functional conservation of vertebrate retinas, and the ongoing capacity for rod neurogenesis throughout the lifetime in zebrafish. A transgenic zebrafish line (XOPS:eGFP) in which rod photoreceptors express green fluorescent protein (GFP) was used as the source of rods. We developed a protocol for the enzymatic dissociation of adult retinal tissue, which successfully separated individual cells. Rod photoreceptors were isolated using a BD Biosciences FACS Aria flow cytometer, based on their expression of GFP. Sorted GFP+ (rods) cells constituted 10-20% of all retinal cells from a single fish. Total RNA was extracted from the sorted GFP+ and GFP- cells, and quality of RNA samples was examined by Agilent 2100 Bioanalyzer. High-quality RNA samples were subsequently amplified and used to construct eight cDNA libraries (4 GFP+ and 4 GFP-) based on samples from four fish, and these were subjected to RNA-seq. Analysis of sequencing data is in progress.

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P346

RETINAL DISTRIBUTION OF DISABLED-1 IN A DIURNAL MURINE RODENT, THE NILE GRASS RAT ARVICANTHIS NILOTICUS

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Purpose: To study the expression pattern of Disabled-1 (Dab1; an adaptor protein in the reelin pathway) in the cone-rich retina of a diurnal murine rodent.

Methods: Expression was examined by western blotting and immunohistochemistry using well established antibodies against Dab1 and various markers of retina neurons.

Results: Western blots revealed the presence of Dab1 (80 kDa) in brain and retina of the Nile grass rat. Retinal immunoreactivity was predominant in soma and dendrites of horizontal cells as well as in amacrine cell bodies aligned at the INL/IPL border. Dab1+ neurons in the inner retina do not stain for parvalbumin, calbindin, protein kinase C- α , choline acetyltransferase, glutamic acid decarboxylase, and tyrosine hydroxylase. They express, however, the glycine transporter GlyT1. They have small ovoid cell bodies ($7.1 \pm 1.06 \mu\text{m}$ in diameter) and bistratified terminal plexii in laminae *a* and *b* of the IPL. Dab1+ amacrine cells are evenly distributed across the retina (2600 cells/mm²) in a fairly regular mosaic (regularity indexes ≈ 3.3 -5.5).

Conclusions: Retinal Dab1 in the adult Nile grass rat exhibits a dual cell patterning similar to that found in human. It is expressed in horizontal cells as well as in a subpopulation of glycinergic amacrine cells undetectable with antibodies against calcium-binding proteins. These amacrine cells are likely of the All type.

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INHIBITION OF THE TGF β SIGNALING PATHWAY INDUCES OVERSHOOTING PROLIFERATION DURING RETINA REGENERATION IN ADULT ZEBRAFISH

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Zebrafish (*Danio rerio*) is an important model organism in eye research. In contrast to mammals, its retina regenerates even after severe damage. During retina regeneration, the TGF-beta signaling pathway is activated. Inhibition of this pathway with chemical inhibitors leads to reduced regeneration in heart and fin. The aim of this study was to analyze retina regeneration in adult zebrafish when the TGF-beta signaling pathway is blocked with a small molecule inhibitor (SB431542). Retina degeneration was induced by placing adult zebrafish in water containing 150 mg/l N-Methyl-N-Nitrosourea (MNU) for one hour. Thereafter, regeneration was assessed by counting the number of cells in each cell layer at day 3, 5, 8, 15, and 30 after MNU treatment. Furthermore, TUNEL staining was performed to detect apoptosis. Most TUNEL positive cells were observed at day 3. Their number was not influenced by the inhibitor until day 5, but thereafter more TUNEL positive cells were observed when the TGF-beta pathway was inhibited. Cell count revealed a slight increase at day 5 and a marked (around 30%) increase of cell nuclei in the inhibited group at day 8 in the outer nuclear layer. Thereafter, cell count decreased to the level of the untreated group. In conclusion, inhibiting the TGF-beta signaling pathway induces overshooting retina regeneration after MNU treatment. This is in contrast to the findings in the zebrafish heart and fin, but recently it has been demonstrated by Lenkowski et al. that an increased activation of the TGF-beta pathway interferes with retina regeneration.

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P348

WHOLE EXOME ANALYSIS IDENTIFIES FREQUENT CNGA1 MUTATIONS IN JAPANESE POPULATION WITH AUTOSOMAL RECESSIVE RETINITIS PIGMENTOSA

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To date, over 40 autosomal recessive retinitis pigmentosa (arRP) causing genes have been reported and several of them (*USH2A*, *EYS*, and *ABCA4*) are known as frequent arRP-causing genes in populations of European descent. However, in the Japanese population, only the *EYS* gene has been reported as a frequent cause of arRP (Hosono et al., *Plos one* 2011, Iwanami et al., *IOVS* 2011). The purpose of this study was to investigate frequent disease-causing gene mutations in arRP in the Japanese population. In total, 101 Japanese patients with non-syndromic and unrelated arRP or sporadic RP (spRP) were recruited in this study and ophthalmic examinations were conducted for the diagnosis of RP. Among these patients, whole exome sequencing analysis of 32 RP patients and direct sequencing screening of all *CNGA1* exons of the other 69 RP patients were performed. As a result, whole exome sequencing of 32 arRP/spRP patients identified disease-causing gene mutations of *CNGA1* (four patients), *EYS* (three patients), and *SAG* (one patient) in eight patients. Screening of an additional 69 arRP/spRP patients for the *CNGA1* gene mutation revealed one patient with a homozygous mutation. In summary, this is the first identification of *CNGA1* mutations in arRP Japanese patients. The frequency of *CNGA1* gene mutation was 5.0% (5/101 patients). *CNGA1* mutations are the most frequent arRP-causing mutations in Japanese patients after *EYS* mutations.

IRB Status: Approved

Disclosures:

SATOSHI KATAGIRI: No financial relationships to disclose

HIGH FIDELITY HUMAN PLURIPOTENT STEM CELLS FOR OCULAR REGENERATIVE MEDICINE

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Cord blood (CB) cells offer an attractive universal donor source for generating human induced pluripotent stem cells (hiPSC) since they carry few somatic mutations, and can more efficiently generate nonviral, clinically relevant pluripotent stem cell lines that could be assembled to create an HLA-defined stem cell bank via worldwide networks of existing repositories. The Zambidis Lab has recently developed and tested clinical-grade non-integrated CB-derived myeloid iPSCs for generating therapeutically important vascular lineages. We have generated highly proliferative embryonic endothelial-pericytic vascular progenitors (VP) from high-fidelity reprogrammed conventional and naïve ground state CB-derived hiPSC lines that can be utilized for treating blinding ischemic eye disease. The regeneration of retinal capillaries with such cellular therapies could potentially reverse the ischemic death of retinal neurons, and ameliorate or prevent end stage blindness in disorders such as diabetic retinopathy and branched vein occlusion. In studies completed this year, we reported that, for the first time, purified hiPSC-derived embryonic VP functionally integrated into ischemia-damaged NOD-SCID mouse retinal vasculature. In contrast to VP differentiated from standard hiPSC lines, embryonic VP from high-fidelity reprogrammed nonviral CB-iPSC possessed lower culture senescence, expanded more robustly in culture, demonstrated more resistance to DNA damage, and were more akin molecularly to those generated from hESC. More importantly, VP generated from CB-iPSC lines possessed an inherent advantage for long-term in vivo survival, migration, homing, and specific engraftment to ischemia/reperfusion-injured retinal tissues. Here, we present work demonstrating the feasibility of generating (in parallel) unlimited supplies of transplantable human embryonic VP along with retinal photoreceptors from patient-specific or HLA-matched naïve ground state hiPSC for a comprehensive regeneration of the damaged retina. These novel human pluripotent stem cell lines, the humanized ocular vascular regenerative model we employ to test the regenerative potential of hiPSC-derived VP, and our collaborators' recent demonstration that our high-fidelity CB-iPSC can generate photoreceptors with a highly advanced degree of maturation, altogether establish important pre-clinical tools for evaluating hiPSC-based therapies to treat blinding ischemic retinopathies.

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AP-2 α AND AP-2 β ARE REQUIRED FOR HORIZONTAL CELL DEVELOPMENT AND AMACRINE CELL MOSAIC PATTERNING

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Previous studies from our lab have shown that the Activating Protein-2 (AP-2) transcription factors, AP-2 α and AP-2 β , are co-expressed in developing horizontal cells and postmitotic amacrine cells. In addition, conditional deletion of both AP-2 α and AP-2 β from the retina (AP-2 α KI/flox/AP-2 β -flox) resulted in anomalies in horizontal and amacrine cells. The neural retina of the AP-2 α KI/flox/AP-2 β -flox mice was further examined in the current study using histological, immunofluorescent and electron microscopy (EM) techniques at embryonic and post-natal stages. Early in development, the double retinal mutants displayed a loss of horizontal cells (HC), as confirmed by the lack of expression of the HC markers, Lim1 and Prox1. Interestingly, the expression of an earlier marker of HC, Onecut-1 (Oc-1), was found to remain intact in the double mutants retina at E16.5, suggesting that these AP-2 genes are downstream of Oc-1. Loss of horizontal cells in the double mutants led to a gradual loss of the outer plexiform layer and caused defects in the triad ribbon synapses of rod photoreceptors, as determined by EM analysis. Although amacrine cells were born in the double mutants, abnormalities in the sublaminae of the inner plexiform layer were observed. These phenotypes were not present in single mutants. Together, these results demonstrate that the expression of AP-2 α and AP-2 β are required for complete differentiation of HC cells and in determining the spatial relationships of amacrine cell processes. These findings further suggest the placement of these AP-2 genes in the genetic cascade required for HC development.

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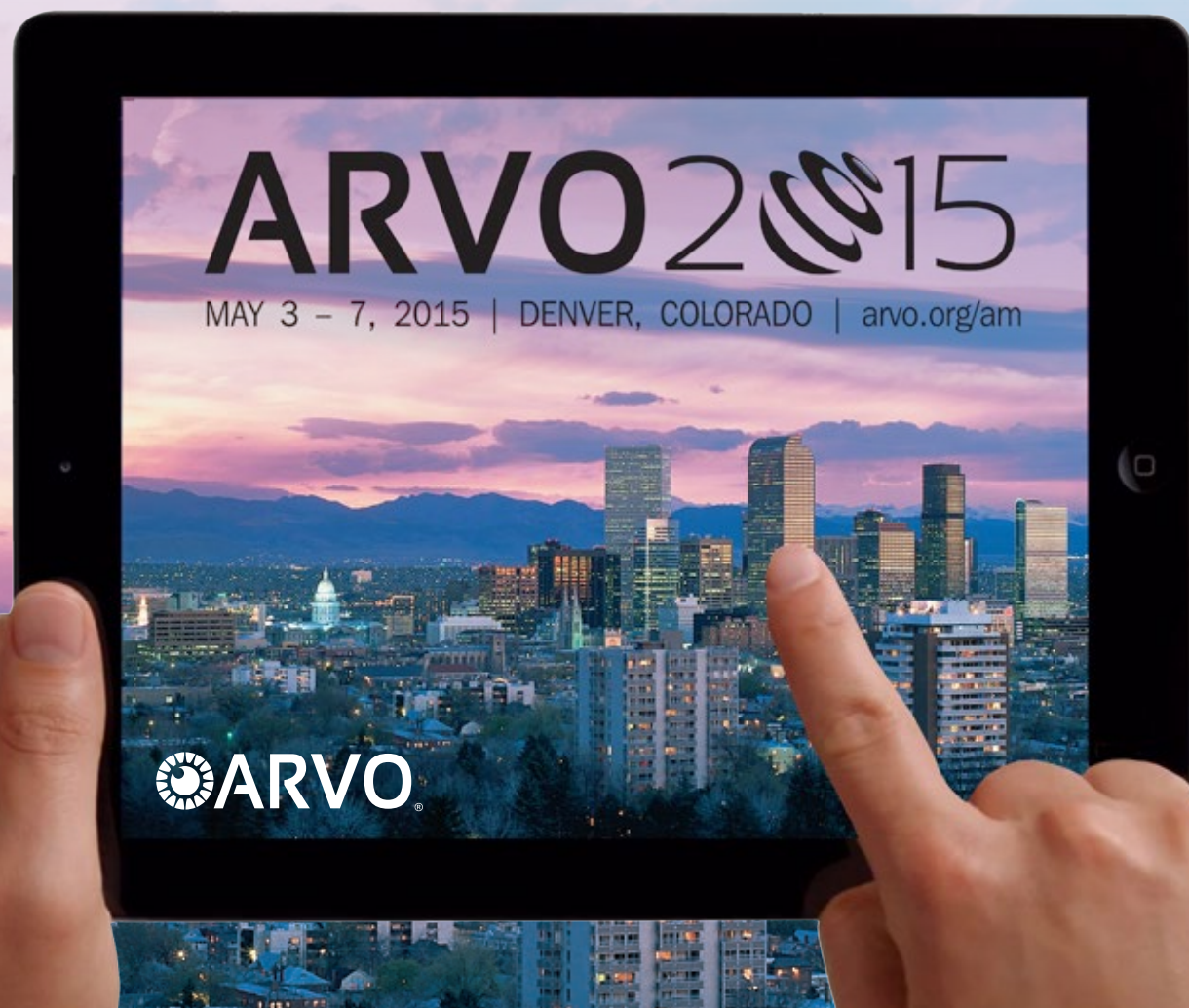
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