International Society
for Eye Research

2013 ISER Sarasota Symposium

Molecular Mechanisms in Glaucoma

September 29-October 2 • Hyatt Regency • Sarasota, FL

Program and Abstract Book

Acknowledgements

The Program Planning Committee and the ISER Council thank the following sponsors for their generous support of the 2013 International Society for Eye Research Sarasota Symposium.

Travel fellowships for young investigators were sponsored by the following companies:

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Welcome

As President, it is my great pleasure to welcome you to the 2013 ISER Sarasota Symposium. This represents the first time in the history of our organization that we have held a topically-focused meeting under the auspices of ISER, as well as having an ISER meeting in an odd-numbered year (our regular Biennial Meetings are held in even-numbered years). After much discussion, the ISER Council decided that the ideal place to hold such a meeting would be Sarasota, FL – a site that most long-time vision and eye researchers associate historically (and with fond memories) with prior ARVO meetings. The focus of this meeting is on molecular mechanisms underlying glaucoma, one of the most clinically significant and prevalent eye diseases world-wide. Hence, this meeting has attracted the attention and attendance of scientists and clinicians from all over the world, including some of the leading experts in the field. Special thanks are due to the dedicated members of the Program Organizing Committee – Drs. Ernst Tamm (Germany), Takeshi Iwata (Japan), Tailoi Chan-Ling (Australia), Rob Nickells (USA), Dan Stamer (USA), and Mike Fautsch (USA) – as well as to Dr. John Penn, ISER's Meeting Liaison, and Ms. Polina Sfard, of San Francisco Association Management Services, Inc. (SF AMS), who spent countless hours helping to coordinate and facilitate this meeting.

ISER's slogan is "making a world of difference in eye research." We specifically make an effort to attract and include Young Investigators (trainees and recently appointed junior faculty) as presenters, not just attendees, in our programs. Also, small meetings of this kind afford and facilitate the ability of graduate and postgraduate students to interact directly with established investigators in their disciplines, to a far greater extent than what is typically achievable in larger scientific and biomedical meetings. ISER also provides Travel Awards to several of our Young Investigator attendees, to facilitate their ability to attend this meeting and present their work.

I especially want to recognize the generous support of our corporate sponsors: BrightFocus Foundation, Heidelberg Engineering, Inc., Novartis, and Santen. Without their support, this meeting would not have been possible.

In closing, I wish you all a very enjoyable and productive meeting. Also – I hope you will make plans to come to the ISER Biennial Meeting in 2014 in San Francisco!

Steven J. Fliesler, PhD
ISER President

Sarasota Welcome Message

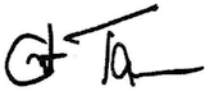
Welcome

On behalf of the Program Planning Committee it is a great pleasure to welcome you to the 2013 International Society of Eye Research Symposium on Molecular Mechanisms in Glaucoma. This symposium will bring together leaders in the field of molecular glaucoma research to discuss the principal molecular mechanisms of this disease. We have generated an exciting program with platform sessions that cover all major aspects of the molecular pathogenesis of glaucoma. We will employ a Gordon Conference format, anticipating that the intimate setting will encourage informal interaction and will offer ample opportunity to refresh or form new collaborations, develop novel hypotheses, and make new friends. The program will feature world-class keynote speakers and hot topics that include: sclera, optic nerve and trabecular meshwork biomechanics, cellular and molecular changes in glaucoma, death and regeneration, extracellular matrix biology, mouse models and many more.

A poster session has been organized to provide a forum for continued and expansive coverage of the topics addressed in the platform sessions, and provide the opportunity for young scientists to present their work and interact with senior specialists.

I trust you will have a memorable and stimulating meeting in Sarasota (just like glaucoma scientists had in old times).

Yours sincerely,



Prof. Dr. Ernst R. Tamm
Program Chair

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About ISER

The Mission of ISER is to provide a unique international platform for discussion and exchange of ideas on contemporary topics in eye and vision research among its members and the broader eye/vision research community.

ISER supports this mission by holding scientific meetings at venues throughout the world, through its journal, Experimental Eye Research, and by focusing resources on the development and support of young investigators, especially those working in the research programs of ISER members.

ISER Membership Information

In June 2013, the Society had over 330 members from approximately 34 countries. The Bylaws of the Society (Article II, Section I) stipulate the following requirements for membership:

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.

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. The Membership Committee will solicit nominations one year prior to the ISER meeting. The Committee will review the nominations and submit recommendations to the Council for action.

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.

Information

All requests for information about membership should be directed to:

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

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Committees

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Travel Fellowships

As part of its commitment to ensure that young investigators from around the world have the opportunity to participate in our meetings, ISER underwrites a Young Investigator Travel Fellowship Program. Based upon established criteria, the ISER Travel Fellowship Committee carefully reviewed and selected 8 travel fellowship awardees from among the many deserving applications. ISER thanks the members of the committee for their dedicated service and congratulates all of those who have received travel awards to the 2013 ISER Sarasota Symposium.

Recipients

Baptiste Coudrillier MS, US
Anitha Krishnan PhD, US
Judy Van Nguyen BS, US
Morgan Rogers BS, US
Uttio Roy Chowdhury PhD, US
Joseph M. Sherwood PhD, UK
Cynthia Von Zee PhD, US
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ISER wishes to thank BrightFocus Foundation, Heidelberg Engineering, Inc., Novartis, and Santen for their support of a Young Investigator Travel Fellowship Awards.

Financial Disclosures

Category	Code	Description
Consultant/Advisor	C	Consultant fee, paid advisory boards or fees for attending a meeting (for the past 1 year)
Employee	E	Employed by a commercial entity
Lecture Fees	L	Lecture fees (honoraria), travel fees or reimbursements when speaking at the invitation of a commercial entity (for the past 1 year)
Equity Owner	O	Equity ownership/stock options of publicly or privately traded firms (excluding mutual funds) with manufacturers of commercial ophthalmic products or commercial ophthalmic services
Patents/Royalty	P	Patents and/or royalties that might be viewed as creating a potential conflict of interest
Grant Support	S	Grant support for the past 1 year (all sources) and all sources used for this project if this form is an update for a specific talk or manuscript with no time limitation

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L – Merck, Sharpe and Dome

Candia, Oscar, MD
P – Mount Sinai School of Medicine, New York, NY

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P – SUNY Downstate

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O – Aerie Pharmaceuticals

Flanagan, John, PhD
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S – Optovue, Inc., Photon etc.

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P – Mt Sinai School of Medicine, New York, NY

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P – Acucela

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C – Amakem, Merck
L – Merck
P – Wisconsin Alumni Research Foundation – WARF

Krizaj, David, PhD
O – Asha Vision, LLC.
P – Asha Vision, LLC.

McLellan, Gillian, BVMS, PhD
C – Ocular Services On Demand (OSOD)

Overby, Darryl, PhD
S – Allergan Inc.

Prestwich, Glenn, PhD
O – ASHA Vision LLC

Ryskamp, Daniel, BS
O – Asha Vision LLC
P – Asha Vision LLC

Schuman, Joel, MD
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Zeilbeck, Ludwig Franz, Dipl. Biol.
Zhang, Lei, MS
Zhang, Lihong, MS
Zhou, Enhua H, PhD
Zhu, Yanli, MD

General Information

Venue

Hyatt Regency Sarasota
1000 Boulevard of the Arts
Sarasota, FL 34236
941-953-1234

Official Language

The official language of the Symposium is English.

Registration Locations and Hours

Registration will be located at the Convention Center Registration Desk, located in the Hyatt Regency Convention Center from September 29–October 2. Any questions about the meeting, posters, and/or social functions may be answered at this location.

Opening Reception

The Opening Reception will be held Sunday September 29, 2013 at 18:00 at the Florida Room and Pool Deck at the Hyatt Regency Sarasota.

Payment of Fees

The International Society for Eye Research accepts cash, checks payable to ISER, MasterCard, and Visa.

Name Badges

All Symposium participants are kindly reminded to wear their name badges throughout the Meeting in order to be admitted to the lecture hall and opening reception.

Certificate of Attendance

Certificate of attendance letters will be available at the Convention Center Registration Desk located in the Hyatt Regency Convention Center, September 29–October 2.

List of Participants

A list of participants is displayed in the registration area.

Information Board

An information board will be located at the Convention Center Registration Desk. Please check regularly for messages and/or important notices.

Liability and Insurance

The Symposium Secretariat and Organizers cannot accept liability or responsibility whatsoever for injury or damage involving persons and property during the Meeting. Participants are advised to take out 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 room.

Smoking Policy

The Hyatt Regency Sarasota is a non-smoking hotel.

Photographing and Taping of Exhibits and Program

No portion of the scientific program or posters may be photographed, audio taped, or videotaped without the written consent of the Society and presenter.

Posters

Posters will be displayed in sections C & D of the Sarasota Ballroom.

Information for Presenters

Data Presentation

Presentations may be prepared for PC or Mac in PowerPoint format. Mac users can also prepare their presentations in Keynote format. There will be a speaker ready station adjacent to the Registration desk located at the Convention Center Registration Desk.

All presentations have to be uploaded at the speaker ready station one hour prior to the presentation time.

Poster Presentations

Posters will be located in Sections C & D of the Sarasota Ballroom. Poster presenters should refer to the list of poster presentations in the program for their board numbers. Your poster may be mounted on Sunday, September 29 between 17:00–19:00. Please remove your poster by 18:00 on Wednesday, October 2. The Organizing Committee will not be responsible for posters that are not removed by end of day, October 2.

Each poster presenter is requested to attend his/her poster for informal discussion during scheduled poster sessions.

Schedule at a Glance

SUNDAY, SEPTEMBER 29

16:00 – 19:00	Check-In, Registration	Convention Center Registration Desk
18:00 – 19:00	Opening Reception	Florida Room and Pool Deck

MONDAY, SEPTEMBER 30

7:00 – 18:30	Registration	Convention Center Registration Desk
8:00 – 8:45	Plenary Lecture I: Claude Burgoyne, MD OPTIC NERVE HEAD CONNECTIVE TISSUE DEFORMATION, DISORGANIZATION AND REMODELING IN EARLY MONKEY EXPERIMENTAL GLAUCOMA — IMPLICATIONS FOR MECHANISMS	Sarasota Ballroom E & F
8:45 – 10:00	Platform Session I: EXTRACELLULAR MATRIX IN GLAUCOMA	Sarasota Ballroom E & F
10:00 – 10:45	Coffee Break/Poster Viewing	Sarasota Ballroom C & D
10:45 – 12:00	Platform Session II: CELL BIOLOGY — NOVEL ASPECTS	Sarasota Ballroom E & F
12:00 – 14:00	Lunch (on own)	
14:00 – 15:15	Platform Session III: PROTECTIVE MECHANISMS	Sarasota Ballroom E & F
15:15 – 16:00	Coffee Break/Poster Viewing with Authors	Sarasota Ballroom C & D
16:00 – 17:15	Platform Session IV: THE ROLE OF GLIA IN GLAUCOMA	Sarasota Ballroom E & F
17:15 – 18:00	Plenary Lecture II: Helmut Kettenmann, PhD THE ROLE OF MICROGLIA IN PATHOLOGY	Sarasota Ballroom E & F

TUESDAY, OCTOBER 1

7:00 – 18:00	Registration	Convention Center Registration Desk
8:00 – 8:45	Plenary Lecture III: Harry Quigley, MD THE ROLE OF THE SCLERA IN GLAUCOMA	Sarasota Ballroom E & F
8:45 – 10:00	Platform Session V: MECHANICAL FACTORS IN GLAUCOMA	Sarasota Ballroom E & F
10:00 – 10:45	Coffee Break/Poster Viewing	Sarasota Ballroom C & D
10:45 – 12:00	Platform Session VI: GROWTH FACTORS	Sarasota Ballroom E & F
12:00 – 14:00	Lunch (on own)	
14:00 – 15:15	Platform Session VII: MECHANOTRANSDUCTION AND BEYOND	Sarasota Ballroom E & F
15:15 – 16:00	Coffee Break/Poster Viewing with Authors	Sarasota Ballroom C & D
16:00 – 17:15	Platform Session VIII: CYTOSKELETON AND CONTRACTILE MECHANISMS	Sarasota Ballroom E & F
17:15 – 18:00	Plenary Lecture IV: Marie-Luce Bochaton-Piallat, PhD BIOLOGICAL PROPERTIES OF THE MYOFIBROBLAST IN HEALTH AND DISEASE	Sarasota Ballroom E & F

WEDNESDAY, OCTOBER 2

7:00 – 18:00	Registration	Convention Center Registration Desk
8:00 – 8:45	Plenary Lecture V: David Epstein, MD IF ONE COULD THERAPEUTICALLY RESTORE OUTFLOW FUNCTION TO NORMAL	Sarasota Ballroom E & F
8:45 – 10:00	Platform Session IX: NEW AND OLD MOLECULES	Sarasota Ballroom E & F
10:00 – 10:45	Coffee Break/Poster Viewing	Sarasota Ballroom C & D
10:45 – 12:00	Platform Session X: REGULATORY PATHWAYS	Sarasota Ballroom E & F
12:00 – 14:00	Lunch (on own)	
14:00 – 15:15	Platform Session XI: CELL DEATH	Sarasota Ballroom E & F
15:15 – 16:00	Coffee Break/Poster Viewing with Authors	Sarasota Ballroom C & D
16:00 – 17:15	Platform Session XII: REGENERATION	Sarasota Ballroom E & F
17:15 – 18:00	Plenary Lecture VI: Simon John, PhD UNDERSTANDING GLAUCOMA MECHANISMS USING MICE	Sarasota Ballroom E & F

Program Schedule

SUNDAY, SEPTEMBER 29

16:00 – 19:00	Check-In, Registration	Convention Center Registration Desk
18:00 – 19:00	Opening Reception	Florida Room and Pool Deck

MONDAY, SEPTEMBER 30

7:00 – 18:30	Registration		Convention Center Registration Desk
8:00 – 8:45	**Plenary Lecture I:** **Claude Burgoyne, MD** **OPTIC NERVE HEAD CONNECTIVE TISSUE DEFORMATION, DISORGANIZATION AND REMODELING IN EARLY MONKEY EXPERIMENTAL GLAUCOMA — IMPLICATIONS FOR MECHANISMS** Introduction by Michael Fautsch		Sarasota Ballroom E & F
8:45 – 10:00	**Platform Session I: EXTRACELLULAR MATRIX IN GLAUCOMA** Moderators: Ernst Tamm and Abbot Clark		Sarasota Ballroom E & F
	8:45 – 9:05	Baptiste Coudrillier (Johns Hopkins), Jacek Pijanka, Joan Jefferys, Harry Quigley, Craig Boote, Thao Nguyen Oral Presentation 1 – AGE AND GLAUCOMA-RELATED CHANGES IN THE MECHANICAL PROPERTIES AND COLLAGEN FIBER STRUCTURE OF THE HUMAN SCLERA	
	9:05 – 9:25	Ted Acott (Casey Eye Institute / Oregon Health & Science University), John Bradley, Mini Aga, Janice Vranka, Kate Keller, Mary Kelley Oral Presentation 2 – SEGMENTAL DISTRIBUTION OF INTRAOCULAR PRESSURE HOMEOSTATIC MECHANISM COMPONENTS	
	9:25 – 9:45	John Kuchtey (Vanderbilt University), Lampros Panagis, Monique McCallister, Rachel Kuchtey Oral Presentation 3 – IMPAIRED AQUEOUS HUMOR OUTFLOW FACILITY IN MICE WITH MICROFIBRIL DEFECTS	
	9:45 – 10:00	Forest Danford (University of Arizona), Weston Welge, Jennifer Barton, Jonathan Vande Geest Oral Presentation 4 – IN-VITRO LAMINA CRIBROSA DEFORMATIONS USING OCT	
10:00 – 10:45	Coffee Break/Poster Viewing		Sarasota Ballroom C & D
10:45 – 12:00	**Platform Session II: CELL BIOLOGY — NOVEL ASPECTS** Moderators: Robert Nickells and Michael Walter		Sarasota Ballroom E & F
	10:45 – 11:05	Michael Elliott (University of Oklahoma), Xiaowu Gu, Nicole Ashpole, Timothy Boyce, Masaki Tanito, Ernst Tamm, Daniel Stamer Oral Presentation 5 – ROLE OF CAVEOLIN-1 IN CONVENTIONAL AQUEOUS OUTFLOW PATHWAY STRUCTURE AND FUNCTION	
	11:05 – 11:25	Nicholas Delamere (University of Arizona), Mohammad Shahidullah Oral Presentation 6 – THE SIGNIFICANCE OF HALF A CHANNEL: CONNEXIN HEMICHANNELS IN THE NONPIGMENTED CILIARY EPITHELIUM	
	11:25 – 11:45	Paloma Liton (Duke University) Oral Presentation 7 – CATHEPSIN B IS UP-REGULATED AND MEDIATES EXTRACELLULAR MATRIX DEGRADATION IN TRABECULAR MESHWORK CELLS FOLLOWING PHAGOCYTIC CHALLENGE	

	11:45 – 12:00	Mary Kelley (Oregon Health & Science University), Xinbo Li, Ted Acott Oral Presentation 8 – ZO-1 ASSOCIATES WITH MULTIPLE PROTEINS IN TRABECULAR MESHWORK AND SCHLEMM'S CANAL CELLS	
12:00 – 14:00	Lunch (on own)		
14:00 – 15:15	**Platform Session III: PROTECTIVE MECHANISMS** Moderators: Rudolf Fuchshofer and John Morrison		Sarasota Ballroom E & F
	14:00 – 14:20	Robert Nickells (University of Wisconsin), Margaret Maes, Joel Dietz, Cassandra Schlamp Oral Presentation 9 – GENETIC ANALYSIS OF SUSCEPTIBILITY TO OPTIC NERVE DAMAGE IN MICE SUGGESTS A PROTECTIVE ROLE FOR AUTOPHAGY	
	14:20 – 14:40	Michael Walter (University of Alberta), Yoko Ito Oral Presentation 10 – DYSFUNCTION OF THE STRESS-RESPONSIVE FOXC1 TRANSCRIPTION FACTOR CONTRIBUTES TO THE EARLIER-ONSET GLAUCOMA OBSERVED IN AXENFELD-RIEGER SYNDROME PATIENTS	
	14:40 – 15:00	Ernst Tamm (University of Regensburg), Ludwig Zeilbeck, Stephanie Leopold, Andreas Ohlmann Oral Presentation 11 – NORRIN INHIBITS THE DEVELOPMENT OF GLAUCOMA IN DBA/2J MICE	
	15:00 – 15:15	Anitha Krishnan (Massachusetts Eye and Ear Infirmary/Harvard Medical School), Alex Jones, Ann Marshak-Rothstein, Bruce Ksander, Meredith Gregory-Ksander Oral Presentation 12 – THE ABSENCE OF SOLUBLE FAS LIGAND RESULTS IN ACCELERATED GLAUCOMA IN DBA/2J MICE	
15:15 – 16:00	Coffee Break/Poster Viewing with Authors		Sarasota Ballroom C & D
16:00 – 17:15	**Platform Session IV: THE ROLE OF GLIA IN GLAUCOMA** Moderators: Mark Johnson and Nicholas Delamere		Sarasota Ballroom E & F
	16:00 – 16:20	Rebecca Sappington (Vanderbilt University School of Medicine), Simone Abella, Lindsay Maxwell Oral Presentation 13 – FORMATION OF MICROGLIA MICROENVIRONMENTS IN RESPONSE TO GLAUCOMA-RELATED STRESSORS	
	16:20 – 16:40	Rudolf Fuchshofer (University of Regensburg) Oral Presentation 14 – REACTIVATION OF ASTROCYTES AND REMODELING OF LAMINA CRIBROSA REGION IS MEDIATED BY CTGF	
	16:40 – 17:00	Tailoi Chan-Ling (University of Sydney) Oral Presentation 15 – UNIQUE PATHOPHYSIOLOGY OF THE OPTIC NERVE HEAD: RELEVANCE TO GLAUCOMA	
	17:00 – 17:15	Jeremy Sivak (University Health Network/University of Toronto), Xiaoxin Guo, John Flanagan Oral Presentation 16 – THE ROLE OF PGC-1ALPHA IN METABOLIC REGULATION OF ASTROCYTE ACTIVATION AND GANGLION CELL INJURY	
17:15-18:00	**Plenary Lecture II:** **Helmut Kettenmann, PhD** **THE ROLE OF MICROGLIA IN PATHOLOGY** Introduction by Tailoi Chan-Ling		Sarasota Ballroom E & F

TUESDAY, OCTOBER 1

7:00 – 18:00	Registration		Convention Center Registration Desk
8:00 – 8:45	**Plenary Lecture III:** **Harry Quigley, MD** **THE ROLE OF THE SCLERA IN GLAUCOMA** Introduction by Robert Nickells		Sarasota Ballroom E & F

8:45 – 10:00	**Platform Session V: MECHANICAL FACTORS IN GLAUCOMA** Moderators: Rebecca Sappington and Daniel Stamer	Sarasota Ballroom E & F
	8:45 – 9:05	Ian Campbell (Georgia Institute of Technology/Emory), William Koh, Johanne Mensah, Hannah Jones, Edward Sander, Julie Albon, Richard Abel, Michael Girard, Ross Ethier Oral Presentation 17 – EFFECTS OF CONNECTIVE TISSUE ORIENTATION ON OPTIC NERVE HEAD BIOMECHANICS IN GLAUCOMA
	9:05 – 9:25	Mark Johnson (Northwestern University), Rocio Vargas-Pinto, Amir Vahabikashi, Kristin Perkumas, Haiyan Gong, Daniel Stamer Oral Presentation 18 – PREFERENTIAL CONTRIBUTION OF FILAMENTOUS ACTIN TO THE STIFFNESS OF THE SCHLEMM'S CANAL CELL CORTICAL CYTOSKELETON
	9:25 – 9:45	Murray Johnstone (University of Washington), Riukang Wang Oral Presentation 19 – BEYOND STATIC IOP: PHS-OCT MEASUREMENT OF PULSE-DEPENDENT MOVEMENT PERMITS EXPLORATION OF FUNCTIONAL PROPERTIES OF TISSUES INVOLVED IN THE GLAUCOMA PROCESS
	9:45 – 10:00	Kate Keller (Casey Eye Institute / Oregon Health & Science University), Ying Ying Sun, Ted Acott Oral Presentation 20 – HYALURONAN SYNTHASE REGULATION AND HYALURONAN CABLE FORMATION BY TRABECULAR MESHWORK CELLS
10:00 – 10:45	Coffee Break/Poster Viewing	Sarasota Ballroom C & D
10:45 – 12:00	**Platform Session VI: GROWTH FACTORS** Moderators: Ted Acott and Paul Kaufman	Sarasota Ballroom E & F
	10:45 – 11:05	Abbot Clark (U. North Texas Health Science Center), Colleen McDowell, Holly Tebow, Robert Wordinger Oral Presentation 21 – TGFB2 USES THE SMAD SIGNALING PATHWAY TO ELEVATE INTRAOCULAR PRESSURE IN THE MOUSE
	11:05 – 11:25	Robert Wordinger (UNT Health Science Center/ North Texas Eye Research Institute), Tara Tovar, Ashley Fitzgerald, Abbot Clark Oral Presentation 22 – INDUCTION OF BIOLOGICALLY ACTIVE BONE MORPHOGENETIC PROTEIN - 1 BY TRANSFORMING GROWTH FACTOR BETA-2 IN HUMAN TRABECULAR MESHWORK CELLS
	11:25 – 11:45	Barbara Braunger (University of Regensburg), Stefan Pielmeier, Cora Demmer, Victoria Landstorfer, Daniela Kawall, Ingo Kleiter, Dietmar Fischer, Herbert Jägle, Ernst Tamm Oral Presentation 23 – TGF-β SIGNALING PROTECTS RETINAL NEURONS FROM PROGRAMMED CELL DEATH DURING DEVELOPMENT
	11:45 – 12:00	Morgan Rogers (Duke University), Iris Navarro, Kristin Perkumas, Craig Crosson, Daniel Stamer Oral Presentation 24 – PEDF DECREASES OUTFLOW FACILITY
12:00 – 14:00	Lunch (on own)	
14:00 – 15:15	**Platform Session VII: MECHANOTRANSDUCTION AND BEYOND** Moderators: Terete Borras and Donna Peters	Sarasota Ballroom E & F
	14:00 – 14:20	John Flanagan (University of Toronto), Ken Olsen, Jeremy Sivak Oral Presentation 25 – GLIAL CELL ACTIVATION FOLLOWING COMPRESSIVE INSULT
	14:20 – 14:40	Enhua Zhou (Harvard School of Public Health), Chan Young Park, Daniel Stamer, Kristin Perkumas, James Butler, Mark Johnson, Jeff Fredberg Oral Presentation 26 – HIGH THROUGHPUT SCREENING FOR DRUGS THAT REDUCE CONTRACTILITY OF SCHLEMM'S CANAL ENDOTHELIAL CELLS
	14:40 – 15:00	Joshua Morgan (UC Davis), Christopher Murphy, Paul Russell Oral Presentation 27 – MODULATION OF YES-ASSOCIATED PROTEIN (YAP) LOCALIZATION IN HUMAN TRABECULAR MESHWORK CELLS IN RESPONSE TO STRESS

	15:00 – 15:15	David Krizaj (University of Utah), Amber Frye, Yong Xu, Glenn Prestwich, Daniel Ryskamp Oral Presentation 28 – NEUROPROTECTION IN GLAUCOMA IS ACHIEVED BY BLOCKING MECHANOSENSITIVE ION CHANNELS IN RETINAL GANGLION CELLS	
15:15 – 16:00	Coffee Break/Poster Viewing with Authors		Sarasota Ballroom C & D
16:00 – 17:15	**Platform Session VIII: CYTOSKELETON AND CONTRACTILE MECHANISMS** Moderators: Stanislav Tomarev and Takeshi Iwata		Sarasota Ballroom E & F
	16:00 – 16:20	Paul Kaufman (University of Wisconsin-Madison) Oral Presentation 29 – MOLECULAR MECHANISMS IN THE REGULATION OF INTRAOCULAR PRESSURE	
	16:20 – 16:40	Donna Peters (University of Wisconsin- School of Medicine & Public Health), Debjani Gagen, Jennifer Faralli Oral Presentation 30 – ACTIVATION OF αvβ3 INTEGRIN INHIBITS PHAGOCYTOSIS AND DECREASES OUTFLOW FACILITY	
	16:40 – 17:00	Vasantha Rao (Duke University) Oral Presentation 31 – RHO GTPASE/RHO KINASE SIGNALING IN HOMEOSTASIS OF AQUEOUS HUMOR OUTFLOW RESISTANCE AND INTRAOCULAR PRESSURE	
	17:00 – 17:15	Terete Borras (University of North Carolina at Chapel Hill), LaKisha Buie, Juan Carabana Oral Presentation 32 – CAUSES AND MECHANISMS OF INHIBITING RHOA IN THE TRABECULAR MESHWORK	
17:15 – 18:00	**Plenary Lecture IV:** **Marie-Luce Bochaton-Piallat, PhD** **BIOLOGICAL PROPERTIES OF THE MYOFIBROBLAST IN HEALTH AND DISEASE** Introduction by Ernst Tamm		Sarasota Ballroom E & F

WEDNESDAY, OCTOBER 2

7:00 – 18:00	Registration		Convention Center Registration Desk
8:00 – 8:45	**Plenary Lecture V:** **David Epstein, MD** **IF ONE COULD THERAPEUTICALLY RESTORE OUTFLOW FUNCTION TO NORMAL** Introduction by Daniel Stamer		Sarasota Ballroom E & F
8:45 – 10:00	**Platform Session IX: NEW AND OLD MOLECULES** Moderators: Tailoi Chan-Ling and Ross Ethier		Sarasota Ballroom E & F
	8:45 – 9:05	Stanislav Tomarev (NIE, NIH), Naoki Nakaya, Afia Sultana Oral Presentation 33 – OLFACTOMEDIN DOMAIN-CONTAINING PROTEINS, OLFM1 AND OLFM2: NEW PLAYERS IN GLAUCOMA	
	9:05 – 9:25	Raquel Lieberman (Georgia Institute of Technology) Oral Presentation 34 – MOLECULAR DETAILS AND CONTROL OF MYOCILIN AMYLOIDOGENESIS	
	9:25 – 9:45	Takeshi Iwata (National Hospital Organization Tokyo Medical Center) Oral Presentation 35 – ENHANCED OPTINEURIN E50K-TBK1 INTERACTION EVOKES PROTEIN INSOLUBILITY AND INITIATES FAMILIAL PRIMARY OPEN-ANGLE GLAUCOMA	
	9:45 – 10:00	John Crabb (Cleveland Clinic), Cheri Stowell, Geeng-Fu Jang, Lei Zhang, Jack Crabb, Belinda Williard, Claude Burgoyne Oral Presentation 36 – QUANTITATIVE PROTEOMIC ANALYSIS OF THE MONKEY OPTIC NERVE HEAD (ONH) IN EARLY EXPERIMENTAL GLAUCOMA (EG)	

10:00 – 10:45	Coffee Break/Poster Viewing	Sarasota Ballroom C & D
10:45 – 12:00	**Platform Session X: REGULATORY PATHWAYS** Moderators: Nicholas Marsh-Armstrong and Vasantha Rao	Sarasota Ballroom E & F
	10:45 – 11:05	Darryl Overby (Imperial College London), Jacques Bertrand, David Woodward Oral Presentation 37 – PHARMACOLOGIC MODULATION OF CONVENTIONAL OUTFLOW FACILITY IN A MOUSE MODEL OF STEROID-INDUCED OCULAR HYPERTENSION
	11:05 – 11:25	Uttio Roy Chowdhury (Mayo Clinic), Cindy Bahler, Bradley Holman, Cheryl Hann, Michael Fautsch Oral Presentation 38 – ATP SENSITIVE POTASSIUM (KATP) CHANNEL OPENERS LOWER INTRAOCULAR PRESSURE BY ACTIVATING THE ERK1/2 SIGNALING PATHWAY IN VIVO
	11:25 – 11:45	Claire Mitchell (University of Pennsylvania), Alan Laties Oral Presentation 39 – LINKING ELEVATION OF INTRAOCULAR PRESSURE TO SUSTAINED CHANGES IN LOCAL INFLAMMATION WITH ATP RELEASE AND THE P2X7 RECEPTOR
	11:45 – 12:00	Joseph Sherwood (Imperial College London), Jason Chang, Alexandra Boussommier-Calleja, Jacques Bertrand, Darryl Overby Oral Presentation 40 – A SIMPLIFIED PUMPLESS PERFUSION SYSTEM FOR MEASURING CONVENTIONAL OUTFLOW FACILITY IN MICE
12:00 – 14:00	Lunch (on own)	
14:00 – 15:15	**Platform Session XI: CELL DEATH** Moderators: Michael Fautsch and Robert Wordinger	Sarasota Ballroom E & F
	14:00 – 14:20	Richard Libby (University of Rochester Medical Center), Kimberly Fernandes, Jeffrey Harder, Peter Shrager, Simon John Oral Presentation 41 – JNK SIGNALING IN AXONALLY INJURED RETINAL GANGLION CELLS
	14:20 – 14:40	Iok-Hou Pang (UNT Health Science Center), Byung-Jin Kim, Robert Wordinger, Richard Libby, Abbot Clark Oral Presentation 42 – IN VITRO AND IN VIVO NEUROPROTECTIVE EFFECTS OF JNK INHIBITORS
	14:40 – 15:00	Cecilia Dong (SUNY Downstate), Konstantin Astafurov, John Danias Oral Presentation 43 – EFFECT OF COMPLEMENT COMPONENT C3 ON RETINAL GANGLION CELLS (RGCS) AND AXONAL LOSS IN A MICROBEAD-INDUCED MOUSE MODEL OF GLAUCOMA
	15:00 – 15:15	Judy Van Nguyen (Johns Hopkins University School of Medicine), Nicholas Marsh-Armstrong, Mark Ellisman, Eric Bushong, Keun-Young Kim, Chung-ha Davis, Akshay Murthy Oral Presentation 44 – MYELIN DEGENERATION AND ASTROCYTE PHAGOCYTOSIS IN GLAUCOMA
15:15 – 16:00	Coffee Break/Poster Viewing with Authors	Sarasota Ballroom C & D
16:00 – 17:15	**Platform Session XII: REGENERATION** Moderators: Darryl Overby and John Danias	Sarasota Ballroom E & F
	16:00 – 16:20	Markus Kuehn (University of Iowa), Qiong Ding, Kristin Anfinson, Emily Kaalberg, Budd Tucker Oral Presentation 45 – INDUCTION OF TRABECULAR MESHWORK CELLS FROM INDUCED PLURIPOTENT STEM CELLS
	16:20 – 16:40	Jeff Gidday (Washington University), Lihong Zhang, Chia-Wen Chiang, Yanli Zhu Oral Presentation 46 – ACTIVATING INNATE EPIGENETIC RESPONSES FOR RETINAL GANGLION CELL PROTECTION IN GLAUCOMA
	16:40 – 17:00	John Danias (SUNY Downstate), Rosana Gerometta, Sandeep Kumar, Shaily Shah, Oscar Candia Oral Presentation 47 – TISSUE PLASMINOGEN ACTIVATOR (TPA) CAN BOTH REVERSE AND PREVENT STEROID-INDUCED IOP ELEVATION

	17:00 – 17:15	Yiqin Du (University of Pittsburgh), Hongmin Yun, Enzhi Yang, Joel Schuman Oral Presentation 48 – POTENTIAL OF EXOGENOUS AND ENDOGENOUS STEM CELL-BASED THERAPY FOR GLAUCOMA
17:15 – 18:00	**Plenary Lecture VI:**	Sarasota Ballroom E & F
	Simon John, PhD **UNDERSTANDING GLAUCOMA MECHANISMS USING MICE** Introduction by Takeshi Iwata	

Oral Presentation Abstracts

Platform Session I: EXTRACELLULAR MATRIX IN GLAUCOMA

Oral Presentation Abstract 1 – AGE AND GLAUCOMA-RELATED CHANGES IN THE MECHANICAL PROPERTIES AND COLLAGEN FIBER STRUCTURE OF THE HUMAN SCLERA

BAPTISTE COUDRILLIER[1], Jacek Pijanka[2], Joan Jefferys[3], Harry Quigley[3], Craig Boote[2], Thao Nguyen[1]

[1]Johns Hopkins University, Baltimore, MD, USA
[2]Cardiff University, School of Optometry and Vision Sciences, Cardiff, Wales, UK
[3]Johns Hopkins University, Baltimore, MD, USA

The objective of this study was to characterize the mechanical properties and collagen fiber structure of 11 sclera from normal donors of age 14-91, and 10 sclera from diagnosed glaucoma donors of age 69-91. Mask evaluations of optic nerve sections were graded to confirm the glaucoma status. Samples were subjected to pressure-controlled inflation tests. Surface displacements were calculated by digital image correlation (DIC). After testing, the collagen fiber structure was measured using wide-angle x-ray scattering (WAXS). We generated specimen-specific finite element meshes based on experimental geometry and thickness. The sclera was modeled as a fiber reinforced material, with collagen fibers dispersed in the ground substance. The model incorporated the details of the WAXS-measured collagen fiber structure. An inverse finite element method was developed to determine the matrix and fiber stiffness by matching model predicted and experimental displacements. The collagen fiber structure and mechanical properties were compared to estimate the effects of age and glaucoma. Among normal specimens, the degree of fiber alignment in the peripapillary sclera decreased with age (p=0.03). Older age was predictive of a larger matrix stiffness (p=0.03), and a non-significant increase in fiber stiffness (p=0.55). In finite element simulations of a full inflation test, we found that scleral canal expansion remained constant with age. The variations in degree of fiber alignment in the peripapillary sclera were different between normal and glaucoma specimens (p=0.03). Glaucoma specimens had a more uniform degree of fiber alignment around the ONH compared with normal specimens. Glaucoma eyes with more than 25% of axon loss (n=4) had a larger matrix stiffness and fiber stiffness, although the differences were not significant (p=0.18 and p=0.58). The observed differences in the biomechanical response of normal and glaucoma sclera may represent baseline properties that contribute to axon damage, or may be characteristics that result from glaucomatous disease.

Oral Presentation Abstract 2 – SEGMENTAL DISTRIBUTION OF INTRAOCULAR PRESSURE HOMEOSTATIC MECHANISM COMPONENTS

TED ACOTT, John Bradley, Mini Aga, Janice Vranka, Kate Keller, Mary Kelley

Casey Eye Institute / Oregon Health & Science University, Portland, OR, USA

Intraocular pressure (IOP) is the primary risk factor for glaucomatous optic nerve damage. IOP is dependent on the aqueous humor outflow resistance, thought to reside within the trabecular meshwork (TM) near Schlemm's canal inner wall endothelium (SCE). IOP homeostasis is a process whereby TM/SCE cells sense pressure changes, probably as mechanical stretch/distortion, and adjust the outflow resistance over several days' time to restore normal IOP. Since outflow is highly uneven around the circumference of the eye, the IOP homeostatic response may also be segmental. Studies were conducted to understand this pressure-dependent homeostatic resistance adjustment and its relative segmental distribution. Matrix metalloproteinase (MMP) activity, an early step in the resistance adjustment process, is greater at normal (1x) perfusion pressures in high flow (HF) regions than in low flow (LF) regions. The 24-72 hour response of increasing from 1x to 2x perfusion pressure or mechanical stretching, which initiates IOP homeostasis, triggers increases in MMP2, MMP14, SPARC, fibronectin, S6 kinases and protein kinase B phosphorylation, podosome- and invadopodia-like structure (PILS) formation, and numerous of other related components. Some of these components are initially more prevalent in HF areas, while others more in LF areas. The segmental distribution of these components after 2x perfusion pressure is mixed. SPARC, a matricellular or organizing protein, which is likely involved in any extracellular matrix remodeling of the outflow resistance, is increased by pressure elevation. It is higher in HF compared to LF regions at 1x perfusion pressure and higher in LF compared to HF regions at 2x pressure. It is still unclear whether the outflow resistance decrease triggered by 2x perfusion pressure occurs in previously HF or previously LF areas. Unraveling the details of this homeostatic resistance adjustment process should be facilitated by comparison of HF and LF components. (NIH # EY003279, EY008247, EY010572)

Oral Presentation Abstract 3 – IMPAIRED AQUEOUS HUMOR OUTFLOW FACILITY IN MICE WITH MICROFIBRIL DEFECTS

JOHN KUCHTEY, Lampros Panagis, Monique McCallister, Rachel Kuchtey

Vanderbilt University, Vanderbilt Eye Institute, Nashville, TN, USA

Based on our identification of a functional variant in a microfibril-associated gene, ADAMTS10, in a dog model of glaucoma, we formed the hypothesis that glaucoma may be caused by microfibril defects. Other microfibril-related genes have previously been identified as causative for glaucoma: LOXL1 in pseudoexfoliation glaucoma and LTBP2 in primary congenital glaucoma. Microfibrils are polymers of fibrilin-1 that provide elastic support in the extracellular matrix and are abundant in the trabecular meshwork and optic nerve head. In addition to their structural function, microfibrils are the primary reservoir of latent transforming growth factor beta (TGFβ). In diseases associated with microfibril defects, TGFβ signaling is hyper-activated and plasma TGFβ concentration is elevated. Defective microfibrils could provide a mechanistic explanation for the established elevation of TGFβ in the aqueous humor of human glaucoma patients. In the present study, we performed experiments with mice carrying a C1059G variant of fibrilin-1 that causes Marfan syndrome, a known microfibril deficiency. Mice heterozygous for the mutation (Fbn1c1059G/+) are known to have disordered microfibril structures, elevated systemic TGFβ and chronic activation of TGFβ signaling in the aortic wall. We found that TGFβ signaling is activated in the trabecular meshwork of Fbn1c1059G/+ mice compared to controls, as indicated by nuclear localization of phosphorylated SMAD2/3. At 6 months of age, Fbn1c1059G/+ mice have reduced facility of aqueous humor outflow (0.0073 +/- 0.0038 µl/min/mmHg, mean +/SD, n=7) compared to controls (0.0146 +/- 0.0067 µl/min/mmHg, n=9, $p<0.05$) as determined by perfusion of enucleated eyes. The activated TGFβ signaling and reduced aqueous humor outflow facility of Fbn1c1059G/+ mice support our hypothesis that defects in microfibril structure or function are a mechanism of glaucoma pathogenesis

Oral Presentation Abstract 4 – IN-VITRO LAMINA CRIBROSA DEFORMATIONS USING OCT

FOREST DANFORD[1], Weston Welge[2], Jennifer Barton[1], Jonathan Vande Geest[1]

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In the majority of glaucoma cases, the mechanism through which the retinal ganglion cells (RGCs) are damaged is unknown. The literature strongly suggests that the primary site of axon death occurs at the lamina cribrosa. As such, the biomechanical properties of the lamina cribrosa are important for determining the environment that RGCs are subject to in the optic nerve head (ONH). Our laboratory is exploring the use of optical coherence tomography (OCT) as a means to map the in-vitro pressure-dependent deformations of the lamina cribrosa. We have utilized a commercially available swept-source OCT system (ThorLabs, Inc.) with an axial resolution of 9 microns, lateral resolution of 11 microns, and total imaging depth of 2 mm. We have preliminary 3D reconstructions of the laminar beams through the entire depth of the ONH at conditions simulating 5, 15, 30, and 45 mmHg of IOP. Initial testing showed a fresh porcine eye had non-linear increases in both the nasal-temporal (N-T) and superior-inferior (S-I) dimensions when changing from lower to higher IOPs. The largest deformation in the N-T dimension was 60 microns going from 15 to 30 mmHg IOP and 66 microns in the S-I dimension when going from 5 to 15 mmHg IOP. Our ultimate goal is optimizing our setup for testing eyes of various ages and ethnicities to ultimately use inverse finite element simulations to determine the lamina cribrosa's material properties. We are also comparing our OCT results to laminar mapping via two photon microscopy (second harmonic imaging), which has a much finer X-Y resolution at the cost of longer imaging times and less depth of penetration. Determination of the mechanical properties of the lamina cribrosa is important for future computational simulations investigating variability in ethnic groups and how these differences may play a role in the onset and/or development of glaucoma.

Platform Session II: CELL BIOLOGY – NOVEL ASPECTS

Oral Presentation Abstract 5 – ROLE OF CAVEOLIN-1 IN CONVENTIONAL AQUEOUS OUTFLOW PATHWAY STRUCTURE AND FUNCTION

MICHAEL ELLIOTT[1], Xiaowu Gu[1], Nicole Ashpole[2], Timothy Boyce[1], Masaki Tanito[3], Ernst Tamm[4], Daniel Stamer[2]

[1]University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA
[2]Duke University, Durham, NC, USA
[3]Shimane University Faculty of Medicine, Izumo, Shimane, Japan
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Gene association studies have linked polymorphisms in the CAV1/2 gene locus to increased risk of primary open angle glaucoma.The proteins encoded by these genes are the signature structural proteins of caveolae, specialized, flask-shaped plasma membrane domains that are abundant in the conventional aqueous outflow pathway that includes Schlemm's canal endothelium and trabecular meshwork cells. In this study, we examined the consequences of caveolin-1 (Cav-1) gene deletion on intraocular pressure, aqueous humor outflow, and outflow pathway morphology and ultrastructure. Global Cav-1 knockout (KO) mice and age/sex-matched controls were used for these studies. Intraocular pressure was measured by rebound tonometry (Tonolab), outflow facility was measured in perfused enucleated eyes using a computerized perfusion system, and outflow tissue morphology was assessed by light and transmission electron microscopy. Intraocular pressure measured in 12-24 week old Cav-1 KO mice was significantly higher than age-/sex-matched controls (15.1 ± 0.5 versus 12.4 ± 0.3 mmHg, $p \leq 0.001$, n = 32 KO and 30 control eyes, respectively). Pressure-dependent outflow facility, measured at 4 sequentially increasing pressure steps (8, 15, 25, 35 mmHg), was significantly reduced by 43% in Cav-1 KO mice compared to controls (0.045 ± 0.012 versus 0.079 ± 0.005 µl/min/mmHg; $p \leq 0.05$, n = 9 KO and 8 control eyes). Ultrastructural analysis revealed a loss of morphologically-identifiable 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 absent. Thus, our results demonstrate pathological consequences on the conventional outflow pathway resulting from the loss of Cav-1 and caveolae. Collectively, these results indicate an important role of Cav-1/caveolae in conventional outflow and intraocular pressure regulation that merits further mechanistic studies. Support: R01EY019494 (MHE), EY022359 (WDS), P30EY021725, BrightFocus Foundation Thomas R. Lee Award for Glaucoma Research (MHE), Alcon Young Investigator Award (MHE), and an unrestricted grant from Research to Prevent Blindness, Inc.

Oral Presentation Abstract 6 – THE SIGNIFICANCE OF HALF A CHANNEL: CONNEXIN HEMICHANNELS IN THE NONPIGMENTED CILIARY EPITHELIUM

NICHOLAS DELAMERE, Mohammad Shahidullah

University of Arizona, Tucson, AZ, USA

The formation of aqueous humor requires the coordinated activity of ion transporters and channels in the pigmented (PE) and nonpigmented ciliary epithelium (NPE). Apical gap junctions constitute functional channels that couple the NPE and PE. Accordingly, connexin 43 (Cx43) is detectable where the two layers meet. Here we report on connexins at the NPE basolateral surface, where the cells face the aqueous humor. In certain tissues unpaired connexons form hemichannels that, when open, connect the cytoplasm with the extracellular space. To examine possible hemichannel function, studies were conducted on intact porcine eyes and porcine NPE established in primary culture. Connexins were detected by immunolocalization and Western blot. Cx43 and Cx50 were observed at the NPE basolateral surface. Entry of propidium iodide (PI) (Mol. wt. 668) and fluorescein dextran (FDex) (Mol. wt 4000), which are membrane impermeable, was examined as an indicator of hemichannel opening. Under control conditions, only trace amounts of PI entered native and cultured NPE. Hemichannel opening is increased by extracellular calcium removal and in calcium-free buffer PI entry increased significantly. Importantly, PI entry was suppressed by 18α glycyrrhetinic acid (100 µM), a gap junction inhibitor. FDex did not enter the cultured NPE either in control or calcium-free medium, consistent with the expected size limit of hemichannel-mediated solute entry. The findings are consistent with a hemichannel conduit capable of delivering biologically active molecules from the NPE cytoplasm to the aqueous humor. Feasibly, substances released from the NPE could signal to tissues in the outflow pathway. Hemichannels raise the possibility of inflow-outflow coupling. Funding: NIH-EY006915.

Oral Presentation Abstract 7 – CATHEPSIN B IS UP-REGULATED AND MEDIATES EXTRACELLULAR MATRIX DEGRADATION IN TRABECULAR MESHWORK CELLS FOLLOWING PHAGOCYTIC CHALLENGE

PALOMA LITON

Duke University, Durham, NC, USA

Cells in the trabecular meshwork (TM), a tissue responsible for draining aqueous humor out of the eye, are known to be highly phagocytic. Phagocytic activity in TM cells is thought to play an important role in outflow pathway physiology. However, the molecular mechanisms triggered by phagocytosis in TM cells are unknown. Here we investigated the effects of chronic phagocytic stress on lysosomal function using different phagocytic ligands (E. coli, carboxylated beads, collagen I-coated beads, and pigment). Lysotracker red co-localization and electron micrographs showed the maturation of E. coli- and collagen I-coated beads-containing phagosomes into phagolysosomes. Maturation of phagosomes into phagolysosomes was not observed with carboxylated beads or pigment particles. In addition, phagocytosis of E. coli and collagen I-coated beads led to increased lysosomal mass, and the specific up-regulation and activity of cathepsin B (CTSB). Higher levels of membrane-bound and secreted CTSB were also detected. Moreover, in vivo zymography showed the intralysosomal degradation of ECM components associated with active CTSB, as well as an overall increased gelatinolytic activity in phagocytically challenged TM cells. This increased gelatinolytic activity with phagocytosis was partially blocked with an intracellular CTSB inhibitor. Altogether, these results suggest a potential role of phagocytosis in outflow pathway tissue homeostasis through the up-regulation and/or proteolytic activation of extracellular matrix remodeling genes. This work was supported by National Institutes of Health Grants R01EY020491, R21EY019137, P30EY005722, Brightfocus Foundation (G2012022) and Research to Prevent Blindness

Oral Presentation Abstract 8 – ZO-1 ASSOCIATES WITH MULTIPLE PROTEINS IN TRABECULAR MESHWORK AND SCHLEMM'S CANAL CELLS

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ZO-1, a tight junction protein, is also associated with gap and adherens junctions. Gap junctions, comprised of connexin (Cx) proteins, act as a diaphragm between cells, allowing passage of selected molecules. Trabecular meshwork (TM) plays a critical role in intraocular pressure (IOP) homeostasis, yet the underlying mechanism is not entirely clear. To facilitate our understanding of gap junctions and IOP, we investigated ZO-1 and associated proteins. Cultured porcine (PTM) and human TM (HTM) cells were maintained as previously described. Porcine Schlemm's canal (SC) cells were isolated with anti-CD31 antibody and magnetic beads. Integrins and afadin were detected by RT-PCR and/or Western immunoblotting, and connexins were identified by immunohistochemistry and confocal microscopy. Co-localization of ZO-1 with other proteins was determined by immunofluorescence and co-immunoprecipitation. Interactions of connexins with PDZ domains of ZO-1 were monitored by the GST pulldown assay. Using the anterior segment perfusion model system, the gap junction blocker carbenoxolone was evaluated for its effect on outflow. Alpha3 and beta1 integrin, as well as afadin, Cx37 and Cx43 were found to be present, but not Cx30.2, Cx36, Cx40, Cx45, or tight junction protein claudin-1 in TM. ZO-1 partially co-localized with alpha3 integrin, afadin, and Cx43, immunoprecipitated Cx43 and alpha3 integrin, and interacted directly with the PDZ1 domain of Cx37 and the PDZ2 domain of Cx43. Carbenoxolone increased outflow over several days. ZO-1 and associated proteins may form a multi-protein complex. Gap junction blockers may facilitate outflow via ZO-1 and the multi-protein complex and/or changing their relationships. (NIH/NEI:#EY008247,EY003279,EY010572)

Platform Session III: PROTECTIVE MECHANISMS

Oral Presentation Abstract 9 – GENETIC ANALYSIS OF SUSCEPTIBILITY TO OPTIC NERVE DAMAGE IN MICE SUGGESTS A PROTECTIVE ROLE FOR AUTOPHAGY

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The Rgcs1 quantitative trait allele on mouse chromosome 5 confers dominant resistance to neuronal death caused by optic nerve damage. 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. Quantitative expression analysis of genes within the Rgcs1 allele, and sequence analysis, implicates a gene called Spink2 as the prime candidate affecting the cell death phenotype. 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 most susceptibility. Spink2 appears to act as a suppressor of autophagy. Cells expressing Spink2 fused to blue fluorescent protein are able to form autophagosomes in response to rapamycin. These cells, however, accumulate an excessive number of LC3-II containing autophagosomes. Maturation of these vesicles into autolysosomes through fusion with lysosomes appears to be disabled by the SPINK2 protein. We hypothesize that the reduction or impairment of a normal autophagy in BALB/cByJ mice is the underlying mechanism leading to increased susceptibility of retinal ganglion cells to optic nerve damage. Supported by NIH R01 EY018869, P30 EY016665, and Research to Prevent Blindness

Oral Presentation Abstract 10 – DYSFUNCTION OF THE STRESS-RESPONSIVE FOXC1 TRANSCRIPTION FACTOR CONTRIBUTES TO THE EARLIER-ONSET GLAUCOMA OBSERVED IN AXENFELD-RIEGER SYNDROME PATIENTS

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FOXC1 mutations are associated with Axenfeld-Rieger Syndrome (ARS), an autosomal dominant disease of the anterior segment of the eye. Structures in the anterior segment of the eye, such as the trabecular meshwork (TM), play an essential role in regulating aqueous humor flow and thus, intraocular pressure (IOP). Approximately 75% of ARS patients with FOXC1 mutations develop earlier-onset glaucoma, a progressively blinding condition that is often associated with increased IOP. Cellular defense mechanisms enable cells of the TM to adapt to a dynamic environment where there is constant exposure to a variety of stresses, including oxidative stress. We hypothesized that compromised ability of TM cells to adapt to stress due to FOXC1 mutations may underlie part of the pathophysiology of earlier-onset glaucoma in ARS patients. We therefore examined the role of FOXC1 in mediating stress response. Our results indicate that FOXC1 is a stress-responsive transcription factor. Exposure of HTM cells to H_2O_2-induced oxidative stress resulted in a significant decrease in FOXC1 RNA and protein levels. Si-RNA knockdown down of FOXC1 resulted in increased cell death under both normal and oxidatively stressed conditions. In addition, levels of the apoptoic markers cleaved PARP-1 and cleaved caspase-7 significantly increased when FOXC1 was knocked down in oxidatively stressed HTM cells. These results reveal that FOXC1 is an anti-apoptotic protein. We also show that FOXC1 mediates the stress response pathway through the regulation of HSPA6, a member of the HSP70 family of molecular chaperones. FOXC1 directly controls HSP6A expression by binding to HSPA6 regulatory elements. Knocking down HSPA6 in HTM cells sensitizes cells to severe oxidative stress, indicating that HSPA6 has a protective function in HTM cells. Thus, dysregulation of protective genes such as HSPA6 in ARS patients with FOXC1 mutations could have a significant role in ARS-associated earlier-onset glaucoma.

Oral Presentation Abstract 11 – NORRIN INHIBITS THE DEVELOPMENT OF GLAUCOMA IN DBA/2J MICE

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Norrin is a secreted signaling molecule that activates the canonical Wnt / β-catenin signaling pathway via binding to frizzled 4 receptors. Recently, we demonstrated that Norrin protects retinal ganglion cells (RGC) against an acute NMDA-mediated damage via an activation of the Wnt / β-catenin signaling pathway in Müller cells. To analyze if Norrin has similar neuroprotective properties on chronic RGC death in glaucoma, 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. In Pax6-Norrin mice, a moderate expression of Norrin mRNA and an activation of the Wnt / β-catenin pathway were detected in the retina by northern blot analysis and immunohistochemistry. In DBA/2J mice an increased intraocular pressure (IOP) with a maximum of 17.8 ± 1.2 mmHg at the age of 9 month 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. Histological analyses of optic nerves showed that in Pax6-Norrin / DBA/2J mice neuronal damage and reactive glial scarring was less severe 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 summary, the transgenic overexpression of Norrin reduces glaucomatous damage in DBA/2J mice most likely by modifying the outflow system and IOP reduction. It is tempting to speculate that the effects of Norrin are mediated by an activation of the Wnt / β-catenin signaling pathway.

Oral Presentation Abstract 12 – THE ABSENCE OF SOLUBLE FAS LIGAND RESULTS IN ACCELERATED GLAUCOMA IN DBA/2J MICE

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Glaucoma is a complex, multifactorial disease characterized by axonal degeneration and retinal ganglion cell (RGC) loss. The evidence indicates that RGCs undergo apoptosis, but the actual mechanism of cell death remains unclear. We reported previously that Fas ligand (FasL) is a pro-apoptotic molecule within the retina that mediates apoptosis of RGCs in a TNFα-induced model of RGC degeneration. However, the membrane form of FasL (mFasL), that is pro-apoptotic, can also be cleaved to release a soluble molecule (sFasL) that is not apoptotic. To further study the function of mFasL and sFasL in glaucomatous degeneration, we backcrossed our FasL knock-in mouse (ΔCS.1) (only expresses mFasL and no sFasL) with DBA/2J mice. Intraocular pressure was monitored in DBA/2J (D2) and DBA/2J x ΔCS.1 (D2.ΔCS) by Tonolab tonometry, revealing no significant difference between these groups of mice thru 12 months of age. However, D2.ΔCS mice displayed accelerated loss of RGCs and nerve fibers (at 5 months), as compared with D2 mice (at 9 months). Moreover, the accelerated loss of RGCs in D2.ΔCS mice was preceded by increased TUNEL staining in the RGC layer (first observed at 3 months) that correlated with an increase in Iba-1 positive cells throughout the RGC layer. By 12 months of age, D2 mice displayed significant loss of only RGCs with all other retinal neurons spared. However, similarly aged D2.ΔCS mice displayed extensive retinal degeneration that included: the RGC, inner plexiform, and inner nuclear layers. This retinal degeneration was dependent upon IOP and only occurred in D2.ΔCS mice with IOP>20mmHg. We conclude that mFasL is neurotoxic during development of glaucoma and that retinal microglia/macrophages are critical mediators of RGC death. Our data also indicate that sFasL is an important neuroprotective molecule that not only prevents RGC death, but also protects other retinal neurons in hypertensive eyes.

Platform Session IV: THE ROLE OF GLIA IN GLAUCOMA

Oral Presentation Abstract 13 – FORMATION OF MICROGLIA MICROENVIRONMENTS IN RESPONSE TO GLAUCOMA-RELATED STRESSORS

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Recent evidence suggests that cytokine signaling induced by glaucoma-related stressors occurs in spatially discreet regions throughout the ganglion cell and nerve fiber layers of the retina. Here we sought to determine whether microglia reactivity similarly occurs in discreet microenvironments. For these studies, we examined the anatomical patterning of microglia and its relationship to reactivity states in healthy murine retina (young C57) and murine retina exposed to glaucoma-related stressors, including normal aging (aged C57), genetic predisposition to glaucoma (young DBA/2) and elevated intraocular pressure (IOP; aged DBA/2). We examined morphological and biochemical hallmarks of microglia reactivity that are shared by all neuropathies, including ramification state, density, hypertrophy and Iba-1 expression. Using immunohistochemistry, quantitative digital microscopy, morphological quantification methods and correlation/regression analyses, we measured the spatial attributes of these reactivity indices and determined their relationship to the anatomical organization of microglia within discreet (200 μm x 200 μm) microdomains throughout the ganglion cell and nerve fibers of the retina. We found that, like cytokine signaling, indices of microglia reactivity are spatially variable in microdomains throughout the mid-central to mid-peripheral retina. In combination, these reactivity indices revealed the presence of microglia microenvironments that: 1) are spatially discreet, 2) have morphological and biochemical characteristics that can be reliably predicted and 3) increase in number and diversity in response to glaucoma-related stressors. Our data suggest that microglia reactivity occurs in the context of microenvironments, which have distinct morphological and biochemical properties that are likely to reflect equally diverse functions.

Oral Presentation Abstract 14 – REACTIVATION OF ASTROCYTES AND REMODELING OF LAMINA CRIBROSA REGION IS MEDIATED BY CTGF

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In primary open angle glaucoma a remodeling process of the extracellular matrix of the lamina cribrosa (LC) could be observed. The remodeling of the LC together with the increased intraocular pressure (IOP) is suspected to provoke an impaired axoplasmatic flow leading to the loss of retinal ganglion cells. The laminar remodeling is thought to be a feedback mechanism of astrocytes to mechanical stimuli like increased IOP. The elevated expression of transforming growth factor (TGF)-β2, which is detected in the optic nerve head (ONH) of glaucomatous eyes, could be one of the mechanisms of the reactiveted astrocytes leading to the ECM remodelling process. We have established an animal glaucoma model by a lens specific overexpression of the connective tissue growth factor (CTGF) with increased IOP and a progressive loss of axons in the optic nerve. In this glaucoma modell we have now the opportunity to analyze the response of astrocytes to increased IOP. We could observe by immunohistochemistry that the astrocytes of laminar region shows an increased GFAP synthesis in the 2 month old CTGF overexpression mice in comparison to the wild type littermates. In those animals the CTGF level was increased within the lamina region, which was accompanied by an increased synthesis of tropoelastin. In vitro studies of the CTGF effect on primary murine ON astrocytes could prove that CTGF lead to a higher migration rate of astrocytes, an indicator for reactivation astrocytes, and to an increased synthesis of ECM proteins. The alteration within the lamina region in the CTGF overexpression mice was attended with changes in the supply with neurotrophic factors in the retrolaminar part of the ON, observed by a dramatic reduction of BDNF within the retrolaminar region. In conclusion we could show that CTGF could contribute to the pathological changes within ONH of POAG patients.

Oral Presentation Abstract 15 – UNIQUE PATHOPHYSIOLOGY OF THE OPTIC NERVE HEAD: RELEVANCE TO GLAUCOMA

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This presentation highlights the unique structural and functional biology that constitutes the mammalian optic nerve head. Multifactorial processes contribute to the pathogenesis of glaucoma, with a recent emphasis on the role of astrocytes and the blood vessels of the ONH. The challenge confronting the field is to understand the changes in the functional capabilities of astrocytes and blood vessels with ageing and how they relate to glaucoma pathology. Our earlier studies investigated changes in astrocyte density, morphology, proliferation and apoptosis occurring in the retina during 'physiological aging'. The density and total number of parenchymal astrocytes in the retina increased between 3 and 9 months of age but decreased markedly between 9 and 12 months. Proliferation of astrocytes was detected at 3 months but virtually ceased beyond that age, whereas the proportion of astrocytes that were TUNEL positive and relative expression of active caspase 3 and endonuclease G increased progressively with aging. In addition, in aged retinas, astrocytes exhibited gliosis-like morphology and loss of Pax2 reactivity. A small population of $Pax2^{+}/GFAP^{-}$ cells was detected in both young adult and aged retinas. The reduction in the availability of astrocytes in aged retinas and other aging-related changes we found may have a significant impact on the ability of astrocytes to maintain homeostasis and support neuronal function in old age (Mansour et.al *Aging Cell* 2008). Further, we investigated age-associated changes in retinal astrocyte connexins. Cx30 was consistently elevated at 22 months compared to younger ages both when associated with parenchymal astrocytes and vascular-associated astrocytes (Mansour et.al *PLoS ONE* 2013). This presentation will also detail our current studies of the changes in astrocytes and blood vessels in the region of the human optic nerve head during 'physiological aging'.

Oral Presentation Abstract 16 – THE ROLE OF PGC-1ALPHA IN METABOLIC REGULATION OF ASTROCYTE ACTIVATION AND GANGLION CELL INJURY

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Glaucomatous optic neuropathy is associated with a complex combination of risk factors, including age, increased intraocular pressure, and vascular disregulation. These stresses converge to damage the optic nerve head (ONH) and metabolically sensitive retinal ganglion cells (RGCs), in association with prominently increased astrocyte reactivity. However, a molecular mechanism has not yet been identified that integrates local tissue responses to these varied insults. We have generated data identifying the transcriptional co-activator, PGC-1α (peroxisome proliferator-activated receptor gamma co-activator 1 alpha), as a potential key mediator of this process. When activated, PGC-1α interacts with transcription factors regulating metabolic, oxidative, and ischemic stress. Through these interactions PGC-1α acts as a master cellular regulator of adaptive energy metabolism. Expression of PGC-1α was analyzed in acute and chronic mouse retinal injury models by quantitative rt-PCR, and in-situ hybridization. Putative PGC-1α target genes, markers of metabolic and oxidative stress, glial reactivity, and RGC death were assessed by rt-PCR, immunofluorescence microscopy, and TUNEL assay, in combination with loss- and gain-of-function techniques. PGC-1α expression was modulated in RGCs and the NFL with age, and in a model of chronic ocular hypertension, as well as following acute metabolic retinal injury. PGC-1α deletion resulted in significantly increased RGC sensitivity and loss, in association with prominent retinal astrocyte reactivity. Conversely, increased activity was protective against oxidative stress and hypoxia in ONH and brain astrocytes. In conclusion, we have identified an important pathway that can integrate pathogenic susceptibility to metabolic and oxidative injury in the inner retina.

Platform Session V: MECHANICAL FACTORS IN GLAUCOMA

Oral Presentation Abstract 17 – EFFECTS OF CONNECTIVE TISSUE ORIENTATION ON OPTIC NERVE HEAD BIOMECHANICS IN GLAUCOMA

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Retinal ganglion cell loss in glaucoma is likely sensitive to peripapillary sclera and lamina cribrosa (LC) biomechanics, including the porosity and orientation of LC connective tissue elements (beams). Previous models have treated the lamina as isotropic and homogeneous or have considered only a few individual LC beams. Here we present a novel pipeline for biomechanical modeling based on either small-angle light scattering (SALS) or micro-computed tomography (μCT) imaging of the optic nerve head (ONH). Fiber orientation in cryo-sectioned LC and peripapillary sclera from normal human banked eyes was measured by SALS (Albon et al, ARVO 2013). Connective tissue was visualized in whole post mortem porcine optic nerve head (ONH) by contrast-enhanced μCT (Ethier et al, ARVO 2012). Preferred fiber orientations in 2D μCT slices were extracted using Fourier transform-based techniques (Sander et al., JBMR Part A, 2009). LC deformation due to scleral canal expansion was computationally modeled in planar slices with and without accounting for connective tissue fiber orientation. Both SALS and μCT scans provided spatially-resolved fiber/beam orientations in LC and peripapillary sclera; μCT data also allowed determination of local LC tissue porosity. SALS-based finite element models accounting for tissue anisotropy showed that IOP-induced strain was highest in the LC (2.4%) but lowest within a thin-band surrounding the scleral canal boundary (1.4%). Replacing the anisotropic sclera with an isotropic equivalent elevated relative LC strain (3.1%). The presence of a circumferential fiber ring in the peripapillary sclera limits LC strain, which is lowest at the scleral canal boundary, a region prone to LC disinsertion and optic disc hemorrhages in glaucoma. SALS and μCT provide powerful datasets to inject information on ONH connective tissue microarchitecture into tractable biomechanical models of the LC and will be useful tools to understand the role of LC and peripapillary scleral biomechanics in glaucoma. Financial Support: Singapore Ministry of Education, Academic Research Funds, Tier 1 (MJAG); Wolfson Royal Society Award (CRE); Georgia Research Alliance (CRE).

Oral Presentation Abstract 18 – PREFERENTIAL CONTRIBUTION OF FILAMENTOUS ACTIN TO THE STIFFNESS OF THE SCHLEMM'S CANAL CELL CORTICAL CYTOSKELETON

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Endothelia of the inner wall of Schlemm's canal (SC) form pores in response to a transcellular pressure drop, likely facilitating aqueous outflow into the canal lumen. Pore density is reduced in glaucomatous eyes. Recent work suggests that the impaired pore formation in glaucoma may be due to altered SC cell stiffness in the subcortical cytoskeleton (Johnson et al., ARVO, 2013). We investigated the contribution of actin microfilaments to the stiffness of SC cells. SC cells were isolated from human eyes (2 normal and 1 glaucomatous) as previously described (Stamer, 1998). Atomic force microscopy measurements were performed on subconfluent cells with pyramidal or spherical (4.5 or 10 μm) tips, and elastic modulus was determined using a modified Hertz model. Our previous work has shown that sharp tips characterize the cortex stiffness while larger, rounded tips characterize the stiffness of the internal cytoskeleton. Cells were separately transduced with an adenovirus delivering an actin filament marker, rAV-LifeAct-TagGFP2 (IBIDI; Verona, WI) to visualize the actin distribution. F-actin depolymerizing agent Latrunculin-A (1 μM) was used to determine the effects on the actin cortex and internal cytoskeleton. SC cells had pronounced cell cortices and stress fibers, both of which were eliminated by Latrunculin-A treatment. Latrunculin-A greatly reduced the elastic modulus of the cortex of normal cells (86% reduction) and glaucomatous cells (77%). Latrunculin-A also significantly reduced the elastic modulus of the subcortical cytoskeleton but the effect was more variable (normal cells: 35-90%; glaucomatous cells 29-65%). F-actin plays a significant role in determining the cellular modulus particularly for the cell cortex. Its contribution to cortex stiffness was similar for normal and glaucomatous cells. However, it has less influence on the subcortical cytoskeleton particularly for glaucomatous SC cells. Acknowledgements: National Glaucoma Research, a program of the BrightFocus Foundation, NIH EY019696 and NIH T32 EY007128.

Oral Presentation Abstract 19 – BEYOND STATIC IOP: PhS-OCT MEASUREMENT OF PULSE-DEPENDENT MOVEMENT PERMITS EXPLORATION OF FUNCTIONAL PROPERTIES OF TISSUES INVOLVED IN THE GLAUCOMA PROCESS

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Aqueous flows from Schlemm's canal (SC) to the aqueous veins by pulsatile mechanisms that slow and finally stop as glaucoma progresses. Gonioscopy-lens induced SC blood reflux also slows and stops as glaucoma progresses, thought to result from stiffening of trabecular tissues. Pressure-reducing drugs such as adrenergics, miotics and prostaglandins enhance pulsatile aqueous outflow and reduce intraocular pressure (IOP). Pulsatile aqueous discharge from SC requires (TM) movement that is in turn dependent on tissue biomechanical properties that become abnormal in glaucoma. The optic nerve head (ONH) becomes abnormal in glaucoma but to explain progressive optic nerve head damage in the presence of normal IOP, some parameter in addition to pressure, such as abnormal ONH tissue biomechanical properties must be involved. However, no in vivo techniques have been available to assess ONH elasticity and compliance. A phase-sensitive OCT system PhS-OCT, was developed in the Wang Laboratory at the University of Washington, a system with nanometer resolution capable of characterizing both TM and ONH pulse-dependent motion. Ex vivo studies in primates demonstrated pulse-dependent TM motion of as much as 4 microns. In vivo study of 20 human eyes demonstrated pulse-dependent TM motion highly correlated with the ocular pulse ($R2^2$=0.996, P<0.0001). TM motion strength, harmonics, velocity and phase lag were characterized. Phase lag was correlated with heart rate but not age. PhS-OCT also successfully measured pulse-induced axial motion of the ONH in 5 humans with mean amplitude of 3.5±0.8μm and a fundamental frequency of 1.2±0.02Hz. ONH motion was negatively correlated with the central retinal artery pulse (100%). Pixel-based OCT is very useful for imaging structure. PhS-OCT measures in vivo motion of TM and ONH tissues, behavior dependent on elasticity and compliance, a reflection of functional rather than structural properties of the tissues. Characterization of these properties may provide new insights into the glaucoma process.

Oral Presentation Abstract 20 – HYALURONAN SYNTHASE REGULATION AND HYALURONAN CABLE FORMATION BY TRABECULAR MESHWORK CELLS

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Levels of hyaluronan (HA), which is synthesized by three HA synthases (HASs), are decreased in the trabecular meshwork (TM) of normal aged eyes and are highly reduced in glaucomatous TM. Moreover, reducing HA levels by numerous methods increased outflow resistance in human anterior segment perfusion culture. In this study, we investigated HAS gene expression, HA concentration and HA structure in TM cells in response to various stimuli that induce remodeling of the extracellular matrix (ECM). HAS mRNA expression was quantified in TM cells in response to TNFα, IL-1α, TGFβ2 and mechanical stretching and differential responses were observed. HAS2 was increased by all cytokine treatments, but slightly decreased by mechanical stretching. HAS1 was increased by TNFα, IL-1α, and mechanical stretch, but TGFβ2 had no effect. HAS3 was only increased by mechanical stretch. By a competitive ELISA assay, HA concentration was increased in the media by all cytokine treatments, but pericellular HA was not significantly affected. Conversely, pericellular HA was reduced by mechanical stretch, but levels in media were unchanged. HA structure was visualized using HA binding protein (HAbp) and confocal microscopy. TNFα-stimulated gene 6 (TSG6), which is associated with HA cable formation, versican, a HA binding proteoglycan, and CD44, a cellular receptor of HA, were also examined. By immunofluorescence, HAbp was found to extensively label pericellular HA. In treated cells, HA also formed large cable-like structures that extended between cells. TSG6 and versican were immunolocalized along the length of some of these HA cables, but CD44 was restricted to the ends where the cables interacted with the cell surface. Our data suggests that HAS gene expression is differentially modulated in response to various treatments that alter aqueous outflow. Moreover, formation of HA cables may stabilize the ECM of the TM through periods of active remodeling.

Platform Session VI: GROWTH FACTORS

Oral Presentation Abstract 21 – TGFB2 USES THE SMAD SIGNALING PATHWAY TO ELEVATE INTRAOCULAR PRESSURE IN THE MOUSE

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Levels of the profibrotic growth factor TGFβ2 are elevated in the aqueous humor and trabecular meshwork (TM) of POAG eyes. TGFβ2 has a number of direct effects on the TM including enhancing extracellular matrix (ECM) deposition and reorganizing the actin cytoskeleton. In vitro studies in cultured TM cells have shown that TGFβ2 activates both Smad and nonSmad pathways, and both pathways are involved in altered ECM metabolism. Transduction of the TM in mouse eyes with an adenovirus vector over-expressing bioactivated TGFβ2 causes ocular hypertension. The purpose of the present study was to determine whether the Smad signaling pathway is essential for this TGFβ2-induced ocular hypertension using Smad3 knockout mice. The expression vector Ad5. hTGFβ2C226/228S was injected intravitreally into one eye of wild type (WT), heterozygous (HET), and homozygous (KO) 129-Smad3tm1par/J mice (n=9-10 per group), while the uninjected contralateral eye of each animal served as the control. IOPs were measured 1-2 times per week for 5 weeks using a TonoLab rebound tonometer. TM fibronectin (FN) levels were assessed by QRT-PCR analysis and by immunohistochemical staining. Statistical differences were determined using one-way ANOVA. TGFβ2 treatment caused statistically significant differences in IOP elevation between the three Smad3 genotypes. IOP exposures (mmHg elevation x days) were: WT 187.7 +/- 23.9; HET 95.6 +/- 24.5; and KO 52.8 +/- 25.2 (WT vs HET $p<0.05$; WT vs KO $p<0.01$). TGFβ2 increased FN immunostaining in WT compared to KO eyes. FN mRNA expression in the TM increased 2.23 +/- 0.24 fold in WT mice and remained unchanged (0.99 +/- 0.19) in KO eyes ($p<0.05$). Smad3 in the TGFβ2 canonical signaling pathway is essential for TGFβ2-mediated FN deposition in the TM and IOP elevation in the mouse eye. Acknowledgement: Supported by NEI/NIH grant EY017374 COI/Financial Disclosure: None

Oral Presentation Abstract 22 – INDUCTION OF BIOLOGICALLY ACTIVE BONE MORPHOGENETIC PROTEIN -1 BY TRANSFORMING GROWTH FACTOR BETA-2 IN HUMAN TRABECULAR MESHWORK CELLS

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There are limited studies on factors that regulate the processing of extracellular matrix (ECM) proteins into their mature form by TM cells. BMP1 is an enzyme responsible for the cleavage and maturation of the ECM cross-linking enzyme lysyl oxidase (LOX). The purpose of this study was to determine whether: (a) cultured human TM cells express BMP1, (b) BMP1 expression is regulated by TGF-β2, (c) BMP1 is biologically active in TM cells and (d) BMP1 regulates LOX activity in TM cells. Primary human TM cells were isolated and subjected to qPCR and Western immunoblotting (WB) for BMP1. BMP1 immunolocalization was performed in TM tissues. BMP1 activity was measured in TM cells treated with TGF-β2 or with a combination of TGF-β2 /UK383367. LOX enzyme activity was evaluated by WB in TM cells treated with BMP1 or with a combination of BMP1/β-aminoprorionitrile (BAPN). Results indicate that human TM cells expressed BMP1 mRNA and protein. Exogenous TGF-β2 increased BMP1 mRNA expression compared to controls ($p<0.05$). ELISA immunoassay of conditioned medium showed that TGF-β2 induced BMP1 secretion compared to controls ($p<0.05$). Lastly, secreted BMP1 stimulated LOX enzymatic activity in TM cells. Thus, BMP1 is expressed in the human TM cells and stimulates LOX enzymatic activity. TGF-β2 induction of BMP1 may be responsible for increased cross-linking of ECM proteins resulting in increased TM stiffness and resistance to ECM degradation. Acknowledgement: Supported by NEI/NIH: EY017374 COI/ Financial Disclosure: None

Oral Presentation Abstract 23 – TGF-β SIGNALING PROTECTS RETINAL NEURONS FROM PROGRAMMED CELL DEATH DURING DEVELOPMENT

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To investigate the role of TGF-β signaling for developmental programmed cell death of retinal ganglion cells, Tgfbr2flox/flox mice were crossed with α-Cre mice to generate a conditional inactivation of TβRII in optic cup-derived cells of the neural retina. As control, Smad7flox/flox; α-Cre mice were used. Apoptotic cell death of retinal neurons was analyzed by TUNEL labeling and an ELISA for free nucleosomes. The number of retinal ganglion cells and their axons was counted and electroretinography was performed. MRNA expression was analyzed by quantitative RT-PCR. Dissociated retinal cell cultures were treated with TGF- β2. Western blot analysis and immunohistochemical staining showed a significant decrease in the amounts of phosphorylated Smad3 in the retina indicating diminished activity of TGF-β signaling in conditional TβRII-deficient mice. During postnatal synaptogenesis, apoptotic death of retinal neurons was significantly increased in TβRII-deficient pups. Similarly, apoptosis increased in retinal progenitors of TβRII-deficient embryos. In contrast, treatment with TGF-β2 inhibited cell death of retinal ganglion cells in dissociated retinal cell cultures, an effect that was blocked upon inhibition of Smad3 phosphorylation. The increase in developmental apoptosis resulted in a significant reduction of retinal neurons in adult animals; an effect that was most pronounced for retinal ganglion cells and resulted in functional deficits as evidenced by electroretinography. In contrast, conditional deletion of the inhibitory Smad7 protein enhanced Smad3 phosphorylation and decreased apoptosis of retinal neurons in embryos and pups. Conditional TβRII-deficient pups expressed lower amounts of nerve growth factor (NGF) in the retina, while higher amounts were observed in conditional Smad7-deficient pups. We conclude that TGF-β signaling protects retinal ganglion cells from apoptotic cell death during development. It is intriguing to speculate that TGF-β signaling may have a similar neuroprotective role for adult retinal ganglion cells in glaucoma.

Oral Presentation Abstract 24 – PEDF DECREASES OUTFLOW FACILITY

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Pigment epithelial-derived factor (PEDF), a protein produced by the retinal pigment epithelium, regulates the permeability of retinal vessels as part of the blood-retinal barrier. As a constituent of aqueous humor (likely secreted from the ciliary epithelia), the role of PEDF in conventional outflow function is unknown. The goals of our study were to examine the effects of PEDF on barrier function in cultured Schlemm's canal (SC) endothelial monolayers and to determine the effects of PEDF on outflow facility in enucleated mouse eyes. Human or porcine SC cells were seeded on Transwell filters at confluence. After two weeks, the filters were treated with media ± purified PEDF. We observed that PEDF increased transendothelial electrical resistance (TEER) in porcine SC cells in a dose-dependent fashion (0.1-3 μg/ml) and at 1 μg/ml stabilized TEER of human SC monolayers over time (24-48 hr). Using an intact model system, eyes from culled C57BL/6 mice were enucleated and cannulated for ex vivo perfusion of PEDF using a computer-controlled perfusion system optimized for mouse eyes. Purified PEDF (0.1μg/ml and 1μg/ml) was perfused at four different pressures (4, 8, 15, 20 mmHg), measuring flow to determine outflow facility (slope of flow/pressure relationship). We observed a 41.6% decrease in outflow facility (0.016 vs. 0.028 μl/min/mmHg, p = 0.011) in eyes perfused with 1 μg/ml PEDF compared to vehicle-perfused controls; and a 22.5% decrease in outflow facility (0.023 vs. 0.027 μl/min/mmHg, p = 0.005) at 100 ng/ml PEDF. In summary, PEDF increased barrier function in both in vitro and in situ models of the inner wall of SC. The 42% decrease in outflow facility represents the greatest acute effect on outflow resistance observed for any substance tested to date. Thus, blockade of PEDF signaling in SC cells may be therapeutically exploited to increase outflow facility in people with ocular hypertension.

Platform Session VII: MECHANOTRANSDUCTION AND BEYOND

Oral Presentation Abstract 25 – GLIAL CELL ACTIVATION FOLLOWING COMPRESSIVE INSULT

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Purpose: To investigate the biomechanical response of human optic nerve head (ONH) astrocytes to compressive insult. Methods: Primary human ONH astrocytes were grown to confluence on pre-stretched, sylastic membranes (Flexcell® Bioflex® plates). DMEM/F12 media containing 10% FBS and 1% pen/strep, was changed twice weekly. Upon reaching confluence the cells were serum deprived for 24 hours and subjected to 12% cyclic compression at 1 Hz using the FlexerCell® Strain System FX-4000 for 2 hours (n=3). Control cells were also serum deprived for 24 hours followed by another 2 hours (n=3). Cell lysates were collected and LC/MS/MS proteomic analysis was performed to determine protein regulation using the LTQ Orbitrap Velos®. Proteins of interest were selected based on statistical significance ($p<0.05$) and fold change ($>\pm1.5$) and analyzed using the Ingenuity Systems' IPA® biomolecular analysis software. Multiplex LASER Bead analysis of the conditioned media was used to confirm the regulation of secreted proteins, predicted by IPA of regulated intracellular proteins. Results: Proteomic analysis discovered 875 unique proteins, 60 of which were determined to play a role in the response of astrocytes to compressive insult. Several proteins and pathways of particular interest were found to be regulated including FLNB (+4.3), TGFB1 (+4.1), and RHOA (+1.6). Proteins previously identified to be regulated by biomechanical stretch were also discovered including ANXA4 (-2.1), S100 (A16, -10.0), and ROA (+1.7). Secreted proteins, predicted by IPA, included TNFa (+2.3), VEGF (+4.4), IL-6 (+3.0), MMP-2 (+3.1), FGF (+1.9) and TGFB1 (+4.2) Conclusion: Several proteins and pathways were identified to be regulated in response to equi-axial compression of human ONH astrocytes. In particular proteins and pathways associated with astrocyte activation were identified including TGFB1, which is associated with extra cellular matrix remodelling, and RHOA and FLNB which are involved with reorganization of the cytoskeleton and cell morphology.

Oral Presentation Abstract 26 – HIGH THROUGHPUT SCREENING FOR DRUGS THAT REDUCE CONTRACTILITY OF SCHLEMM'S CANAL ENDOTHELIAL CELLS

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Purpose: Outflow resistance in the conventional outflow pathway has been intimately linked to the stiffness and contractility of Schlemm's canal (SC) endothelial cells (Zhou et al. 2011, Journal of the Royal Society, Interface. DOI: 10.1098). We recently developed high throughput traction microscopy (HTTM) and used it to screen the Prestwich library of 1,200 FDA-approved drugs with the goal of finding new bronchodilators which relax the human airway smooth muscle (HASM) cells. Here we applied HTTM to discover drugs that relax the SC cell monolayers. Methods: Human SC cells were isolated from one human donor eye as previously described (Stamer et al. 1998, IOVS); we cultured human SC endothelial cell monolayers on polyacrylamide gels that are coated with type 1 collagen and labeled with fluorescent beads. Traction forces were measured in 96-well plates using HTTM. The initial round of screening was performed on HASM cells using 4-drug mixtures. We then decomposed the cell-relaxing mixtures into 72 single drugs and evaluated their impact on SC cell contractility. Our positive controls included isoproterenol and Y27632, which we have previously shown to relax the SC cells. Results: We identified 17 drugs that relaxed the SC cells. 9 hits were β2-adrenergic receptor agonists, which we have previously shown to relax SC cells. 1 hit was a toxin. The mechanisms by which the other 7 drugs relaxed the SC cells remain unclear and are being investigated. Conclusions: We have developed a high throughput platform based on a physiological endpoint, cell contractility. In a partial screening of the Prestwick library, we have discovered several drugs that relaxed the SC cells. HTTM may be useful for conventional outflow-targeted drug discovery. Acknowledgements: Donor eyes were provided by NDRI and Ontario Eye Bank (Toronto Division). This study was supported by R01EY019696-01 (MJ, WDS, JJF).

Oral Presentation Abstract 27 – MODULATION OF YES-ASSOCIATED PROTEIN (YAP) LOCALIZATION IN HUMAN TRABECULAR MESHWORK CELLS IN RESPONSE TO STRESS

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A primary driver of glaucoma progression is reduction in outflow facility through the human trabecular meshwork (HTM). Reports have documented glaucoma to be associated with decreased cellularity in the HTM, suggesting healthy cell populations may maintain HTM function and outflow facility. The mechanisms of cell loss are poorly understood but oxidative stress has been implicated. A treatment effective at increasing facility, selective laser trabeculoplasty (SLT), is thought to work through focal heating of the HTM as well as other mechanisms. SLT leads to a transient increase in HTM cell proliferation, however, the mechanisms underlying this are not understood. Yes-associated protein (YAP) is a pro-proliferative transcriptional coactivator expressed in the HTM. The transcriptional activity of YAP depends on its localization in either the nucleus (active) or the cytoplasm (inactive). However, the impact of YAP on HTM cell proliferation is unknown. In this study, we determined YAP localization in HTM cells after H_2O_2 treatment and heat shock to model oxidative challenge or the focal temperature during SLT, respectively. HTM cells from 4 donors were maintained in serum free conditions for 3-5 days. The cells were then either treated with 0 or 250μM H_2O_2 for 1 hour or placed at 37°C or 44°C for 2 hours. The cells were immediately fixed and stained for YAP. Nuclear localization was quantified as the contrast between the nuclear and peri-nuclear staining intensity (results given as mean±SEM). Nuclear contrast decreased to 32±10% after H_2O_2 treatment and increased to 190±13% after heat shock. This suggests YAP localization may mediate the changes in HTM cellularity seen in glaucoma and or after SLT. Future research will focus on inducing nuclear localization of YAP as a potential glaucoma therapeutic. Research supported by the National Institutes of Health (R01EY019475, R01EY019970, P30EY12576) and unrestricted funds from Research to Prevent Blindness. COI/ Financial Disclosure: None.

Oral Presentation Abstract 28 – NEUROPROTECTION IN GLAUCOMA IS ACHIEVED BY BLOCKING MECHANOSENSITIVE ION CHANNELS IN RETINAL GANGLION CELLS

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Few viable clinical neuroprotective strategies are available in glaucoma treatment partly because the mechanotransduction mechanism responding to elevated intraocular pressure (IOP) within retinal ganglion cells (RGCs) remains unknown. We have identified TRPV4 channels as novel mechanosensing targets in RGCs, characterized their sensitivity to pressure, developed new TRPV4 antagonists and established their effectiveness in a mouse model of glaucoma. Pressure sensitivity of endogenous mechanosensitive channels in RGCs was determined in dissociated cells and wholemount preparations using optical imaging and high sped pressure clamp. 10 - 50 mm Hg steps of positive pressure evoked dose-dependent cation currents in voltage-clamped RGCs. Likewise, biomechanical strain generated by membrane swelling or substrate stretch induced dose-dependent $[Ca^{2+}]$RGC elevations. Mechanosensitive currents and $[Ca^{2+}]$i signals were suppressed by TRPV4 antagonists HC 067047 and YX001-012. TRPV4 channels were localized to the large majority of RGCs using antibodies validated in KO mice, with prominent immunolabeling of primary dendrites, the prelaminar optic region and the optic nerve head. Consistent with a role in RGC excitability, TRPV4 agonists induced >100-fold increase in the frequency of spontaneous RGC firing measured with multielectrode arrays. Sustained TRPV4 stimulation generated calcium overloads that induced RGC apoptosis under in vitro and in vivo conditions whereas photoreceptors, bipolar and amacrine neurons were spared. TRPV4, but not TRPV1, blockers antagonized RGC apoptosis, induced by increased membrane stretch. Finally, sustained exposure to TRPV4 antagonists blocked pressure induced RGC degeneration in a mouse model of glaucoma. In summary, biophysical, molecular and physiological approaches show a novel, intrinsic, mechanosensitive mechanism that appears to play a central role in RGC excitability, calcium homeostasis and survival. We developed new, selective, TRPV4 antagonists that protect RGCs under in vitro and in vivo conditions. Thus, TRPV4 channels represent a natural target in glaucomatous neuroprotection.

Platform Session VIII: CYTOSKELETON AND CONTRACTILE MECHANISMS

Oral Presentation Abstract 29 – MOLECULAR MECHANISMS IN THE REGULATION OF INTRAOCULAR PRESSURE

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The regulation of intraocular pressure involves contraction and relaxation of the trabecular meshwork (TM), Schlemm's canal inner wall (SCIW) and the ciliary muscle (CM) working together to regulate aqueous outflow. This process may include a mechanical homeostatic regulation of outflow facility in which the TM and SCIW act as a self-coordinated functioning organ in response to mechanical deformation or biochemical, hormonal or metabolic alterations in the surrounding environment. The outflow regulatory system contains macrobiomechanics/ mechanosensitivity elements that react to physical stimuli such as shear stress, distortion by the pressure differential between the anterior chamber and SC, tissue stiffening, cardiac cycle fluctuations, etc., and signaling elements (such as eNOS/ NO) that mediate their reflexive/regulatory responses to physical stressors. Additionally, TM /SCIW endothelia possess cytoskeleton/cell contractility components that act as effectors to control conventional outflow. Uveoscleral outflow may normally be a constant, steady-state but "tunable" when needed waste disposal system. The focus of the presentation will be the molecular bases and interplay of these systems, which allow TM/ SCIW and uveoscleral outflow mechanisms to work together as one functional unit to maintain or alter resistance to flow and IOP, as well as their pathophysiologic and therapeutic implications for glaucoma.

Oral Presentation Abstract 30 – ACTIVATION OF αvβ3 INTEGRIN INHIBITS PHAGOCYTOSIS AND DECREASES OUTFLOW FACILITY

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Integrins play a vital role in many of the biological processes central to the maintenance of aqueous humor outflow and intraocular pressure. These processes include matrix deposition, phagocytosis and contractility. Despite the importance of integrins, we know very little about how integrins function in the trabecular meshwork (TM). In this study we examined how the activation state of αvβ3 integrin may affect TM functions. Prior studies have shown that activation of αvβ3 integrin stimulates cytoskeletal rearrangements which lead to the formation of cross-linked actin networks (CLANs) frequently observed in glaucomatous tissues and glaucomatous and glucocorticoid-treated TM cell cultures (Filla et al., IOVS 52:2952-2958. 2011). Using immortalized cell lines that overexpress either the wildtype β3 subunit or a constitutively active β3 subunit, we found that the constitutive activation of an αvβ3 integrin pathway reduced phagocytosis by ~47% compared to cells overexpressing the wildtype β3 subunit. To determine if activation of αvβ3 integrin impaired outflow facility, porcine organ cultured anterior segments (POCAS) were perfused with an activating β3 antibody (AP-5). The contralateral anterior segment was perfused with an isotype matched control IgG. After 30 min of perfusion with an antibody, eyes were perfused with media alone. Out of the 6 pairs, 5 anterior segments perfused with AP-5 showed a statistically significant decrease in outflow facility compared to IgG control after baseline correction. The decrease ranged from 13.5% to 44% 24hrs after the antibody treatment with an average decrease of 25% (p=0.0079). By light microscopy, the POCAS perfused with the activating AP-5 antibody did not show any major structural alterations. TM cells remained attached along the intact beams. In summary these studies suggest that changes in the repertoire of integrin signaling events are likely to affect aqueous humor outflow. Work was supported by NIH-NEI grants (EY017006, EY0020490, P30 EY016665). COI/ Financial Disclosure: None

Oral Presentation Abstract 31 – RHO GTPASE/ RHO KINASE SIGNALING IN HOMEOSTASIS OF AQUEOUS HUMOR OUTFLOW RESISTANCE AND INTRAOCULAR PRESSURE

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Primary open-angle glaucoma is the second leading cause of blindness in the United States and is commonly associated with diminished aqueous humor (AH) drainage. Although the elevation of intraocular pressure (IOP) resulting from increased resistance to AH outflow through the trabecular meshwork (TM) and Schlemm's canal (SC) is considered to be a primary risk factor for open angle glaucoma, the etiological mechanisms responsible for increased resistance to AH outflow are largely unknown. In a series of experimental studies we have investigated the potential role of Rho/Rho kinase signaling in regulation of aqueous humor outflow and intraocular pressure in a mechanistic manner in both perfusion studies with enucleated eyes and live animal models. While perfusion of Rho kinase inhibitors increases AH outflow facility, the activation of Rho GTPase signaling in AH outflow pathway increases resistance to AH drainage leading to elevated IOP. Our ongoing mechanistic studies have started unraveling the influence of RhoA activity on trabecular meshwork cell plasticity and fate transition into myofibroblasts in serum response factor (SRF) and myocordin-related transcription factor (MRTF)-dependent manner. Significantly, sustained activation of Rho GTPase signaling in outflow pathway not only increased IOP in live rodents but also associated with increased expression of collagen-1, fibroblast specific protein-1 and alpha-smooth muscle actin in TM cells indicating activation of fibrogenic activity. Importantly, the Rho kinase inhibitors suppressed the RhoA, TGF-beta2, connective tissue growth factor (CTGF) and lysophosphatidic acid (LPA) induced contractile and fibrogenic activities in TM cells. Collectively, these studies demonstrate several novel insights into the significance of Rho/Rho kinase signaling in homeostasis of AH outflow resistance and IOP by regulating the TM tissue material properties, extracellular matrix production, cell plasticity, myofibroblast activation and fibrogenic activity. COI/Financial Disclosure: None Funding Support: National Eye Institute/NIH (R01EY018590)

Oral Presentation Abstract 32 – CAUSES AND MECHANISMS OF INHIBITING RHOA IN THE TRABECULAR MESHWORK

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RhoA is a GTP-binding protein that regulates cytoskeletal-dependent cell functions. RhoA activates Rho kinase (ROCK) which in turn phosphorylates MLC and inhibits myosin phosphatase. The RhoA/ROCK signaling pathway is known to induce stress-fiber formation, affect TGFβ, and regulate mineralization in vascular smooth muscle and mesenchymal stem cells. We and others showed that inhibiting the RhoA/ ROCK pathway lowers IOP. To develop a gene therapy, longer-duration treatment of elevated IOP, we generated viral vectors carrying dominant-negative RhoA (dnRhoA), whose expression yields an inactive RhoA (Thr19Asn). In earlier proof of concept experiments, we perfused human organ cultures with adenovirus Ad.dnRhoA2 and obtained an increase in outflow facility (MolVis 8,32,2002). Currently, we have generated self-complementary AAV2 (scAAV2) and treated Wistar rats with a single dose (5X1011 vg). 37 rats were injected OS with scAAV2.dnRhoA while OD received scAAV2.GFP or left untreated. Normotense rats did not show changes from baseline IOP (9.3±0.1 mmHg) (n=8). However, in reverse light-cycle experiments, OSs injected with scAAV2.dnRhoA did not exhibit the nocturnal IOP elevation of their contralateral ODs (n=29 rats). Effect lasted 4-8 weeks. Day IOPs of a representative group were 9.2±0.2 mmHg (OS) vs 9.2±0.2 mmHg (OD). In contrast, night IOPs of the same animals were 9.6±0.4 mmHg (OS) vs 14.3.2±0.4 mmHg (OD) (n=6, p=0.001). To investigate other than stress-fiber reduction dnRhoA IOP-lowering mechanisms, we examined its effect on mineralization. In a DEX-induced calcification model in HTM primary cells, AdhRhoA2 infection reversed the levels of calcification marker ALP (alkaline phosphatase) by 2.9-fold (normalized to genomic DNA, $p<0.01$). In a second BMP2 calcification model, the ROCK inhibitor Y27632 reduced the normalized ALP 4-fold and dissipate the calcification nodules stained with Alizarin Red. We conclude that dnRhoA precludes night IOP elevation in rats. We propose that one of the mechanisms responsible for dnRhoA lowering IOP is that of reducing mineralization.

Platform Session IX: NEW AND OLD MOLECULES

Oral Presentation Abstract 33 – OLFACTOMEDIN DOMAIN-CONTAINING PROTEINS, OLFM1 AND OLFM2: NEW PLAYERS IN GLAUCOMA

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Retinal ganglion cell (RGC) dysfunction and death may be caused by excytotoxic damage by elevated intracellular Ca^{2+} levels. Calcium-permeable AMPA receptors (AMPARs) are expressed in RGCs and play a role in Ca^{2+} homeostasis. Olfactomedin 1 (Olfm1) and Olfactomedin 2 (Olfm2) are secretory glycoproteins that are preferentially expressed in brain and RGCs during development and in adults with Olfm1 being expressed at significantly higher level than Olfm2. Deletion of a 52 amino acid long region in the N-terminal part of Olfm1 led to a decrease in the number of RGCs in homozygous mice. In 14 month-old Olfm1 mutant mice, the amount of Brn3 positive cells was reduced by 40% when compared with wild-type without any changes in intraocular pressure. The axon number and an average axon diameter were reduced in 8 month-old Olfm1 mice when compared with wild-type littermates. The visual evoked potential response was delayed in Olfm1 mutant mice and the amplitude was reduced in Olfm2 knockout mice when compared with wild-type littermates. Shotgun proteomics revealed 59 candidate proteins that co-precipitated with wild-type or mutant Olfm1 proteins in postnatal day 1 brain. Olfm1-binding proteins included GluR2, an AMPAR subunit. Subsequent experiments showed that Olfm1 and Olfm2 interact with GluR1-4 subunits of AMPARs while mutated Olfm1 showed reduced binding to GluR1-4. Modification of mutated Olfm1 interaction with binding targets led to an increase in intracellular Ca^{2+} concentration and activation of ERK1/2, MEK1 and CaMKII in the Olfm1 mutant when compared with their wild-type littermates. Our data suggest that interaction of Olfm1/2 with AMPARs may lead to the modulation of the receptor activity in the retina and changes in intracellular Ca^{2+} levels. A deletion in Olfm1 leads to morphological and physiological changes in the eyes of Olfm1 mutant resembling normal tension glaucoma. COI/Financial Disclosure: None

Oral Presentation Abstract 34 – MOLECULAR DETAILS AND CONTROL OF MYOCILIN AMYLOIDOGENESIS

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Mutations in the olfactomedin domain of myocilin (myoc-OLF) are the strongest genetic link to primary open angle glaucoma. Disease-causing myocilin variants accumulate intracellularly instead of being secreted to the trabecular meshwork (TM), which is toxic to TM cells. Our lab recently provided the first biophysical evidence for the hallmarks of amyloid fibrils within aggregated myocilin. We have now dissected the structural details of myoc-OLF when it is primed for fibrillization. Under mildly destabilizing conditions, wild-type myoc-OLF has a distinct non-native structure that leads to facile fibrilization. Disease-causing variants access this non-native structure under physiological conditions, leading to accelerated growth rates in vitro. At least two peptide stretches within myoc-OLF are responsible for the disparate fibril morphologies observed, which are preferentially accessed by disease variants. A variety of different categories of stabilizing molecules inhibit myoc-OLF fibril growth. Taken together, our results suggest a novel protein-based hypothesis for glaucoma pathogenesis and corresponding therapeutic strategies for further testing in clinically-relevant samples. COI/Financial Disclosure: None. Funding: NIH R01EY021205

Oral Presentation Abstract 35 – ENHANCED OPTINEURIN E50K-TBK1 INTERACTION EVOKES PROTEIN INSOLUBILITY AND INITIATES FAMILIAL PRIMARY OPEN-ANGLE GLAUCOMA

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Optineurin (OPTN) is a multifunctional protein and its E50K mutation responsible for primary open angle glaucoma (POAG) and amyotrophic lateral sclerosis (ALS). We have previously reported our E50K transgenic mouse (E50K-tg, Chi et al., Hum Mol Genet 2010) exhibits thinner retina and retinal ganglion cell loss, while the underlying molecular mechanisms have been unclear. Together with additional phenotypes of E50K-tg retina and over expression studies, the endogenous OPTN and E50K mutant protein dynamics was investigated by iPS Cell (iPSC) from E50K-POAG patients to elucidate the fundamental pathoetiology (Minegishi et al., Hum Mol Genet 2013). For further phenotypic analysis, E50K-tg retina were examined by using anti-GFAP, anti-OPTN, and anti-HA antibodies. The intracellular localization and protein property of endogenous OPTN in wild type control and in E50K carrier were also examined by iPSCs and iPSC-derived neural cells. To elucidate the precise protein dynamics, FLAG-tagged OPTN and E50K were expressed in HEK293T cells and protein complex formation/oligomerization was examined by Native-PAGE. Anti-FLAG immunoprecipitates were obtained for identification of binding partners by LC-MS/MS proteomics. Interaction with obtained candidates and further biochemical analysis were examined by general molecular biological techniques. E50K-tg retina exhibited significant reactive gliosis which is one of the hallmarks of neurodegenerative diseases including glaucoma. In E50K-tg retina, E50K mutant protein is accumulated in OPL where exhibited the severe cell death and atrophy. Overexpressed OPTN and E50K exhibited different hydrophobicity and only E50K is accumulated in endoplasmic reticulum (ER) prior to Golgi transition. These distinct protein properties were also consistent with under endogenous condition in iPSCs and iPSC-derived neural cells from E50K-POAG patient. Mammalian cell-based proteomics revealed distinct complex formation between OPTN and E50K. The treatment with specific inhibitor for E50K specific binding partner rescued the aforementioned abnormal hydrophobicity of E50K mutant. These results indicate the E50K-POAG originates from the alteration of protein affinity that deteriorates the OPTN solubility. This alteration is the probable fundamental cause of other downstream E50K phenotypes such as Golgi deformation, intracellular transport failure and eventually cell deaths.

Oral Presentation Abstract 36 – QUANTITATIVE PROTEOMIC ANALYSIS OF THE MONKEY OPTIC NERVE HEAD (ONH) IN EARLY EXPERIMENTAL GLAUCOMA (EG)

JOHN CRABB[1], Cheri Stowell[2], Geeng-Fu Jang[1], Lei Zhang[1], Jack Crabb[1], Belinda Williard[1], Claude Burgoyne[2]

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John W Crabb[1,2,3], Cheri Stowell[4], Geeng-Fu Jang[1,2,] Lei Zhang[1,2,] Jack S Crabb[1,2,] Belinda Williard[2,] and Claude Burgoyne[4]. [1]Cole Eye Institute and [2]Lerner Research Institute, Cleveland Clinic, Cleveland, OH 44195; [3]Depts of Ophthalmology and Molecular Medicine, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University, Cleveland, OH 44106; [4]Devers Eye Institute, Legacy Research Institute, Portland, OR, United States. To understand the molecular mechanisms underlying neural and connective tissue remodeling in early monkey EG, we quantified proteomic change in the EG eye of 4 monkeys with laser-induced, early unilateral EG (10-30% orbital optic nerve axon loss). ONH tissues from the EG and control eye of each monkey were isolated by trephined punch, protein was extracted in detergent, quantified by amino acid analysis, and digested with trypsin. Peptides were labeled with iTRAQ tags, mixed, fractionated by strong cation exchange chromatography, and analyzed by LC MS/MS with an Orbitrap Elite mass spectrometer. Proteins were identified using the Swiss-Protein human database and iTRAQ tags quantified using code written in R. Proteins quantified with ≥ 2 unique peptides/protein in ≥ 3 monkeys were considered significantly altered if average ratios of treated vs control tissues were above or below the mean ≥ 1 standard deviation (SD) and with p values ≤ 0.05. Over 1000 proteins were quantified in the ONH specimens from 3 or more animals. Among proteins significantly altered in early EG by 2 SD were increased levels of F-actin capping proteins, exocyst complex component 1, tropomyosin alpha-3 and decreased levels of myelin protein P0, cell adhesion molecule 2 and paladin. Altered proteins may contribute to abnormalities in cell adhesion, cytoskeletal/cell-cell/cell-matrix interactions and signaling in the ONH. Overall, the results provide new insights into the molecular mechanisms underlying early glaucomatous damage to the ONH tissues and potential targets for systemic biomarkers for the conversion from ocular hypertension to early ONH disease. [Supported in part by NIH grants EY021840 (JWC), EY022134 (JWC), EY011610 (CFB), Research to Prevent Blindness (RPB) Challenge Grant, RPB Senior Investigator Award, Legacy Good Samaritan Foundation, the Alcon Research Institute and the Cleveland Clinic].

Platform Session X: REGULATORY PATHWAYS

Oral Presentation Abstract 37 – PHARMACOLOGIC MODULATION OF CONVENTIONAL OUTFLOW FACILITY IN A MOUSE MODEL OF STEROID-INDUCED OCULAR HYPERTENSION

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Corticosteroid therapy causes ocular hypertension in susceptible individuals that may lead to secondary glaucoma and vision loss if not treated. In this study, we investigate the efficacy of conventional outflow drugs in a mouse model of steroid-induced ocular hypertension (SI-OHT), focusing on the EP4 receptor agonist 3,7-dithia PGE1 and the prostamide FP receptor agonist bimatoprost that increase conventional outflow facility (C) in human eyes. SI-OHT was induced by systemic dexamethasone (DEX) delivery by implanted minipump in 14 C57BL/6 mice (3 mg/kg/d). Rebound tonometry confirmed IOP elevation from 12.6 ± 1.4 mmHg (mean ± SD) 3-4 days prior to implantation to 16.1 ± 2.4 mmHg after 22-24 days ($p = 0.00003$). Following euthanization, eyes were enucleated and placed within a computerized perfusion system to measure C. One eye from each DEX-treated mouse was perfused with 10 nM 3,7-dithia PGE1 ($N = 5$ eyes) or 1 µM bimatoprost ($N = 7$) while the contralateral eye was perfused with vehicle ($N = 6$ or 8). 3,7-dithia PGE1 had no effect on C in DEX-treated mice (0.011 ± 0.003 vs. 0.012 ± 0.003 µL/min/mmHg, $p = 0.3$), despite increasing C by 56 ± 21% in a parallel study of naïve mice ($N = 5$, $p = 0.024$). Bimatoprost, in contrast, increased C in DEX-treated mice from 0.012 ± 0.005 to 0.026 ± 0.019 µL/min/mmHg ($p = 0.036$). These data suggest that stimulation of the prostamide FP receptor, but not the prostanoid EP4 receptor, may be a promising strategy for lowering C and IOP in steroid induced glaucoma.

Oral Presentation Abstract 38 – ATP SENSITIVE POTASSIUM (KATP) CHANNEL OPENERS LOWER INTRAOCULAR PRESSURE BY ACTIVATING THE ERK1/2 SIGNALING PATHWAY IN VIVO

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Previous studies from our laboratory have shown that KATP channel openers lower pressure in ex vivo human anterior segment perfusion cultures by activating the ERK1/2 signaling pathway. In the current study, we evaluated in vivo the role of ERK1/2 signaling in regulating intraocular pressure (IOP) following treatment with KATP channel openers. In wild type C57BL/6 mice (retired breeders, age >7 months, weight approximately 30 g), one eye was treated daily for 5 consecutive days with KATP channel openers diazoxide (5 mM) and nicorandil (5 mM) alone, and in the presence of U0126 (a specific inhibitor of the ERK1/2 signaling pathway). The fellow eye received vehicle control (DMSO). IOP (average of 1, 4 and 23 hr post-treatment measurements) decreased by 14.9 ± 3.8% in diazoxide ($n=10$, $p=0.002$; range 7.2-18.2%) and 16.9 ± 2.5% in nicorandil ($n=10$, $p=0.002$; range 12.9-18.9%) treated eyes with an absolute IOP reduction of 2.6 ± 0.7 mmHg (range 1.2-3.2 mmHg) for diazoxide and 2.8 ± 0.4 mmHg (range 2.1-3.2 mmHg) for nicorandil. Addition of U0126 inhibited the IOP lowering effect of diazoxide and nicorandil, returning IOP to baseline levels within 72 hours. Histologic assessment of the mouse anterior chamber ocular tissues showed no morphological changes between treated and control eyes. Treatment of normal human trabecular meshwork (NTM) cells with KATP channel openers showed increased actin cytoskeleton reorganization and inhibition of LPA-mediated cellular contraction. Together, these results show that KATP channel openers diazoxide and nicorandil lower IOP in vivo by activating the ERK1/2 signaling pathway. In vitro studies suggest that IOP reduction by KATP channel openers may occur through cellular relaxation by modulation of the actin cytoskeleton. COI/Financial Disclosure: None Supported in part by National Institutes of Health Research Grant EY 21727; the Mayo Foundation; and Research to Prevent Blindness [Lew R. Wasserman Merit Award (MPF) and an unrestricted grant (Department of Ophthalmology, Mayo Clinic)]

Oral Presentation Abstract 39 – LINKING ELEVATION OF INTRAOCULAR PRESSURE TO SUSTAINED CHANGES IN LOCAL INFLAMMATION WITH ATP RELEASE AND THE P2X7 RECEPTOR

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Understanding how elevation of intraocular pressure (IOP) produces long-term changes in cells of the optic nerve head is a major challenge in the study of glaucoma. We propose here that signaling by extracellular ATP may link the mechanical strains induced by increased IOP with an enhanced parainflammatory state in glaucoma. Throughout the body, mechanical strain leads to the efflux of ATP; this extracellular ATP can then stimulate adjacent P2X7 receptors to modulate the cytokine expression and release. This process also occurs in the optic nerve head. ATP release is associated with IOP elevation in numerous chronic and acute models. In the Controlled Elevation of IOP model of transient IOP elevation, the expression of cytokines IL-6 and IL-1beta was increased, but this rise was blocked by P2X7 receptor antagonists. Mechanical stretch of optic nerve head astrocytes leads to an efflux of ATP through pannexin hemichannel pores. Stretch of these astrocytes also leads to autostimulation of P2X7 receptors, elevation of cellular calcium and an upregulation in the expression of cytokines IL-6 and Il-1beta. Cytokine release from these astrocytes was triggered by both stretch and stimulation of the P2X7 receptor. Together these observations suggest that elevation of IOP leads to a mechanosentitive release of ATP from optic nerve head astrocytes and the upregulation and release of cytokines following stimulation of the P2X7 receptor. This ATP release/P2X7 receptor stimulation thus provides a pathway by which mechanical strain can be translated into a more sustained parainflammatory response in glaucoma.

Oral Presentation Abstract 40 – A SIMPLIFIED PUMPLESS PERFUSION SYSTEM FOR MEASURING CONVENTIONAL OUTFLOW FACILITY IN MICE

JOSEPH SHERWOOD, Jason Chang, Alexandra Boussommier-Calleja, Jacques Bertrand, Darryl Overby

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Mouse models are useful for glaucoma research, but measuring conventional outflow facility (C) in mice is technically difficult due to the low flow rates required (~100 nL/min). Consequently, only a handful of laboratories are capable of measuring C in mice, but these measurements would likely become more widespread if the technical hurdles were reduced. In this study, we developed a simplified perfusion system for mouse eyes so as to measure C without a syringe pump, which is the most technically demanding, expensive and most troublesome aspect of mouse eye perfusion. Our pumpless perfusion approach uses a constant-height reservoir to provide a fixed pressure source, a pressure transducer to measure intraocular pressure (IOP), and a state-of-the-art thermal flow sensor (Sensirion SLG64) to measure the flow rate into the eye (Q) with nanoliter per minute resolution. The simplified perfusion system was benchmarked against the tried-and-tested syringe pump perfusion system, using an artificial mouse eye, comprising a 76 µm capillary in parallel with a compliant tube, giving the same resistance (~50 mmHg/µL/min) and compliance (0.02 µL/mmHg) of a typical mouse eye. At each of 6 pressure steps between 4 and 24 mmHg, after steady state was reached, 10 minutes of acquisition were averaged to give mean IOP and Q values. C was calculated as the slope of the Q-vs-IOP relationship. Both systems predicted C to be 0.019 µl/min/mmHg. However, the average time to reach steady state was 45 seconds for the simplified perfusion system, compared to 450 seconds for the syringe pump system. This 10-fold reduction in settling time allows for more pressure steps, improved statistical power, and higher throughput for mouse eye perfusions. Combined with the improved simplicity and less expensive equipment, our pumpless perfusion system represents a notable improvement upon the syringe pump perfusion technique.

Platform Session XI: CELL DEATH

Oral Presentation Abstract 41 – JNK SIGNALING IN AXONALLY INJURED RETINAL GANGLION CELLS

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Axonal injury is a critical insult to retinal ganglion cells (RGCs) in glaucoma. The molecular pathways triggered by glaucomatous axonal insult and how they lead to degeneration are poorly defined. Previously we have shown that Jun N-terminal kinase (JNK) was activated in RGC axons after controlled optic nerve crush (CONC) and that JNK2 and 3 were important regulators of RGC death. Of the multiple kinases that can activate JNK, dual leucine kinase (Dlk) is known to be activated by axonal injury and regulate cell death triggered by pathological JNK activation in neurons. Consistent with published reports (Welsbie DS et. al., and Watkins TA et. al, PNAS, 2013), Dlk deficiency significantly attenuated the number of dying RGCs after CONC ($cCASP3^+$ cells/mm^2: Dlk+/+, 289.9±12.0; Dlk-/-, 30.2±6.5). Dlk deficiency lessened RGC death 35 days following axonal injury similar to the protection observed with retinal deficiency of the canonical JNK substrate Jun (RGC number as % control: WT, 30.4±3.8%; Dlk-/-, 76.9±1.9%; Jun-/-, 69.4±5.0%). Western blot analysis showed that Dlk deficiency attenuated phosphorylation of JUN (pJUN) following CONC. Consistent with there being less pJUN, there was significantly less pJNK in RGC somas in Dlk deficient mice 5 days after CONC. However, pJNK was still present in RGC axons both proximal and distal to the site of injury in Dlk deficient mice. Preliminary analysis of axon function after CONC showed that Jnk2/3 deficiency but not Dlk deficiency attenuated axonal degeneration (compound action potential measurements 7 days after CONC). Thus, JNK signaling may contribute to both somal and axonal degeneration with distinct upstream kinases controlling JNK activation in each cellular compartment. JNK signaling is activated in the DBA/2J mouse glaucoma model. Given the importance of JNK signaling after axonal insult in RGCs, we are currently testing if Jnk2/3 deficiency prevents RGC degeneration in DBA/2J mice.

Oral Presentation Abstract 42 – IN VITRO AND IN VIVO NEUROPROTECTIVE EFFECTS OF JNK INHIBITORS

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The c-Jun N-terminal kinase (JNK) has been shown to be involved in various insults that cause apoptotic death of retinal ganglion cells (RGC). We tested if JNK inhibitors have protective effect on RGC both in vitro and in vivo. For in vitro studies, cytotoxicity was induced in primary cultured adult rat retinal cells by glutamate, TNFα, or by the removal of selective trophic factors. RGC survival was assessed by counting Thy-1-positive cells. For in vivo studies, two models were used: (1) retinal ischemia/reperfusion (I/R) was induced in female C57BL/6J mice by raising the intraocular pressure (IOP) to 120 mmHg for 60 min; and (2) acute ocular hypertension to mimic acute glaucoma attack was induced in rats by raising IOP to 45 mmHg for 7 h. At various time points after the insults, retinal thickness, cell density in the ganglion cell layer (GCL), and retinal functions were evaluated. JNK inhibitors, SP600125 and TAT-JNKi-III, dose-dependently and significantly ($p<0.05$) protected against glutamate- and trophic factor withdrawal-, but not TNFα-induced cultured RGC cell death. In the I/R model, phosphorylation of JNK and cJun in the retina was significantly ($p<0.05$) increased at 1 h after injury. I/R injury significantly ($p<0.05$) decreased the thickness of retinal layers, especially the inner plexiform layer (-38±7%), and inner nuclear layer (-25±7%), cell numbers in the GCL (-30±6%), and ERG b-wave amplitudes (-67±7%). All these changes were protected by SP600125 treatment (5, 15, 30 mg/kg, ip, qd)($p<0.05$). Acute ocular hypertension also decreased GCL cell counts (-51%±8%), which was protected by SP600125 (15 mg/kg, ip, qd)($p<0.001$). Theses results demonstrated involvement of the JNK pathway in retinal degeneration in both in vitro and in vivo models. The neuroprotective effect of JNK inhibitors may represent a useful approach for the potential treatment of certain retinopathies.

Oral Presentation Abstract 43 – EFFECT OF COMPLEMENT COMPONENT C3 ON RETINAL GANGLION CELLS (RGCs) AND AXONAL LOSS IN A MICROBEAD-INDUCED MOUSE MODEL OF GLAUCOMA

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Complement activation has been implicated in glaucomatous neurodegeneration. We attempted to determine the effect of the absence of C3 on retinal ganglion cells (RGCs) and optic nerves (ONs) in a mouse model of microbead-induced glaucoma. Ten-week old male wildtype (WT) and C3 KO mice were used. Animals were subjected to uniocular microbead-induced intraocular pressure (IOP) elevation while the contralateral eye received a small amount of beads that did not cause IOP elevation. Injections were repeated after 3 weeks. Some animals also received lipopolysaccharide (LPS) subcutaneously 1 week prior to the initial microbead injection. Additional groups of naïve mice as well as LPS-treated mice were studied. IOP was monitored twice a week for a total of 8 weeks. RGCs were retrogradely labeled with Fluorogold prior to sacrifice and counted in flat mounts. ON sections were obtained and scored semiquantitatitvely for degenerating axons while in some axonal counts were performed. IOP was significantly higher in microbead-injected eyes compared to contralateral control eyes ($p<0.05$). Microbead-treated eyes (n=21) of WT mice had more severe ON damage than contralateral control eyes, as well as eyes (n=12) of naïve mice, and eyes (n=6) of mice receiving LPS (ANOVA $p<0.00019$). In contrast, microbead-treated eyes of C3 KO (n=20) mice had no significant difference in ON damage from contralateral control eyes, but were different from naïve control eyes (n=4) (ANOVA $p< 0.0028$). Microbead-treated eyes (n=17) of WT mice had no significant difference in RGC counts from contralateral control eyes, LPS treated (n=3) or naïve eyes (n=2) (ANOVA $p>0.37$). Similarly, microbead-treated eyes (n=19) of C3 KO mice had no significant difference in RGC counts from contralateral control eyes (ANOVA $p> 0.97$). In conclusion, microbead injections successfully increased IOP in all eyes treated. Absence of C3 eliminated the difference in IOP-related damage in the ON, without affecting RGC survival.

Oral Presentation Abstract 44 – MYELIN DEGENERATION AND ASTROCYTE PHAGOCYTOSIS IN GLAUCOMA

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Glaucoma is marked by gradual loss of retinal ganglion cells and their axons. We study the molecular changes that occur in the optic nerve head (ONH) of mouse glaucoma model DBA/2J. Our recent findings suggest that disease involves increased instability of myelin membranes and the consequent phagocytosis of these myelin membranes by astrocytes. Using a degeneration-specific antibody against Myelin Basic Protein (dMBP), we quantified a significant fluorescence increase in the ONH of DBA/2J mice, as compared to the control strain DBA/2J-Gpnmb$^+$. In addition to intensity, the morphology of dMBP differed. The change in fluorescence and morphology was most evident at the myelination transition zone (MTZ). Coupled with data from electron microscopy, we concluded that there is increased myelin degeneration at the optic nerve head in DBA/2J. We previously described phagocytic activity by astrocytes in the optic nerve and now show that myelin is one of the materials internalized. To better study myelin internalization, we cultured cortical astrocytes from wildtype mice and incubated them with purified bovine myelin. These cultured astrocytes also internalized myelin, as detected by antibodies to myelin before and after cell membrane permeabilization. Surprisingly, myelin degeneration and evidence of phagocytic astrocytes are not restricted to diseased DBA/2J optic nerve. They can also be found in wildtype mice and aged DBA/2J without axon loss. Our data indicate that the MTZ of the optic nerve is a region that contains less stable myelin and more phagocytic astrocytes than distal optic nerve. This instability increases with glaucoma progression, as does astrocyte reactivity. These findings provide insight into a possible homeostatic mechanism that contributes to the health of the optic nerve, as well as identifies a process that may be affected early during glaucoma progression. Author's COI/Financial Disclosure: None.

Platform Session XII: REGENERATION

Oral Presentation Abstract 45 – INDUCTION OF TRABECULAR MESHWORK CELLS FROM INDUCED PLURIPOTENT STEM CELLS

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Purpose: Loss or dysfunction of trabecular meshwork (TM) cells has been associated with the development of pathologically elevated IOP and it is conceivable that replacement of damaged TM cells could restore function to the TM. Autologous transfer avoids immune responses and the ethical concerns associated with the use of embryonic stem cells. To be clinically viable harvest of the cells must be reliable, minimally invasive, and yield a significant number of cells. We propose that the use of TM-like cells derived from induced pluripotent stem cells (iPSC) created from a patient's own dermal fibroblasts offers the best solution to this challenge. Here we demonstrate that mouse iPSCs can be induced to differentiate into TM like cells suitable for autologous transplantation. Methods: Directed induction of stem cell differentiation was achieved through co-culture of mouse iPSCs with human fetal TM cells for up to 21 days. The resultant TM-like cells (iPSC-TM) were characterized morphologically, immunohistochemically, and functionally. Results: iPSC-TM closely resemble cultured human TM cells morphologically and begin to express many markers of TM cells while ceasing to express pluripotency markers such as SSEA1. Functionally, these cells develop the ability to phagocytose particles. Finally, exposure to dexamethasone (100 nmol) or Phorbol 12-myristate acetate (TPA, 25 ng/ml) causes a distinct increase in the production and secretion of myocilin or MMP3, respectively. Conclusion: Our data demonstrate that iPSCs can be induced to assume a phenotype that resembles native TM cells in many important aspects. These cells not only represent a valuable research tool, but transplantation into glaucomatous eyes with elevated IOP may also restore function to the TM resulting in permanently reduced IOP.

Oral Presentation Abstract 46 – ACTIVATING INNATE EPIGENETIC RESPONSES FOR RETINAL GANGLION CELL PROTECTION IN GLAUCOMA

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Purpose: The ability to achieve transient reductions in acute retinal ischemic injury by adaptive pre- or post-conditioning is well-established. In a mouse model of inducible, open-angle glaucoma, we recently showed that a 2-wk period of repetitive hypoxic preconditioning prior to IOP elevation provided sustained protection against the subsequent loss of retinal ganglion cell (RGC) soma and axons (Zhu Y et al., Mol Med 2012). In the present study we assessed whether repetitive hypoxic postconditioning (RH-Post), initiated after disease onset, would also be protective. Methods: Baseline visual function assessments (flash VEP and optokinetics) were obtained in adult male C57Bl/6 mice prior to sustained elevations in IOP (Tonolab) by episcleral vein suture occlusion. Three days after IOP elevation, animals were randomized to either RH-Post (1 h systemic hypoxia [11% oxygen] every other day), or normoxic control. Visual function was re-assessed after 3 wks of glaucoma, followed by sacrifice for quantifying RGC soma (NeuN in flat mounts) and axon (SMI32 in postlaminar optic nerve cross-sections) survival. Results: Controls exhibited deficits in visual function, and loss of both RGC soma and axons (all endpoints $p<0.05$ vs. fellow eye). In contrast, in RH-Post mice, although IOP remained unchanged, visual function decrements were robustly improved, and extent of soma and axon loss robustly reduced (all endpoints $p<0.05$ vs. controls). Conclusions: These results demonstrate that activating innate epigenetic responses, even after disease onset, abrogates both functional and morphologic indices of glaucomatous RGC injury. Targeting innate, pleiotropic RGC-protective responses represents a novel therapeutic strategy for glaucoma, worthy of further mechanistic and translational study. Support: NIH EY18607 (JMG, YZ), AHAF NGF (JMG, YZ), EY02687 (Dept of Ophthalmology, Washington University), the HOPE Center for Neurological Disorders (Washington University), and the Spastic Paralysis Research Foundation of the Illinois-Eastern Iowa District of Kiwanis International.

Oral Presentation Abstract 47 – TISSUE PLASMINOGEN ACTIVATOR (TPA) CAN BOTH REVERSE AND PREVENT STEROID-INDUCED IOP ELEVATION

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Tissue plasminogen activator is a protease that regulates the coagulation system. It is downregulated in steroid induced glaucoma. We investigated whether tPA can be used therapeutically in this form of glaucoma. One eye of two sheep that had been subjected to bilateral steroid-induced IOP elevation, was injected intravitreally with 100ul of human recombinant tPA (100ug) while the contralateral eye received vehicle containing L-arginine. An additional 4 animals received intravitreal tPA uniocularly concurrently with the initiation of bilateral steroid administration. Animals were sacrificed 11 days after tPA administration and the eyes were enucleated. Tissues were extracted and used to determine the mRNA levels of endogenous PLAT and PAI1. IOP decreased by an average of 9.7 (+ 1.6)mmHg within 24 hours in the steroid pretreated eyes. Eyes concurrently treated with steroid and tPA showed similar IOP patterns to their contralateral eyes for the first 4 days after tPA administration but subsequently did not developed higher IOPs. IOP differed by an average of 11.8 (+ 1.3) mmHg 8 days after tPA administration. In all treated eyes, IOP remained low until sacrifice. Levels of PLAT mRNA in the trabecular meshwork were decreased in tPA treated eyes compared to contralateral controls ($p<0.001$) while levels of PAI1 mRNA remained similar ($p>0.29$). In conclusion recombinant human tPA is effective in both preventing and reversing steroid-induced IOP elevation in sheep. Together with findings in a mouse model of steroid-induced changes in outflow facility as well as published human data these findings suggest that tPA may be useful as a therapeutic agent in steroid-induced glaucoma. Supported by R01 EY020670 and Research to Prevent Blindness Inc.

Oral Presentation Abstract 48 – POTENTIAL OF EXOGENOUS AND ENDOGENOUS STEM CELL-BASED THERAPY FOR GLAUCOMA

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Elevated intraocular pressure (IOP) and aging are the most important risk factors for most forms of glaucoma. Reduced cellularity within the trabecular meshwork (TM) is observed in aging and glaucomatous conditions and is associated with elevated IOP. We previously described that stem cells from TM (TMSCs) are multipotent with the ability to differentiate into phagocytic TM cells; TM stem cells can home to the TM after introduced into mouse anterior chamber (AC). In current study, we examine the potential of exogenous TMSCs to home to laser damaged TM region and of endogenous TMSCs to be activated after laser damage. Mouse TM was damaged by 532 nm laser photocoagulation. Human TMSCs were injected into the mouse AC after laser. Laser only and fibroblast injection served as controls. To detect endogenous stem cell response to laser damage, thymidine analogue 5-Chloro-2′-deoxyuridine (CldU) was given to newborn mice by intraperitoneal injection. After an 8-week chase period, the TM was damaged by laser and 5-Iodo-2′-deoxyuridine (IdU) was injected intraperitoneally. Expression of CldU, IdU and cell proliferation markers was detected by immunostaining. Injection of human TMSCs into the mouse AC reduced IOP while injection of fibroblasts increased IOP. Injected TMSCs were able to home to the damaged TM region. In contrast, fibroblasts were detected in the TM, cornea and iris. Some injected TMSCs maintained stem cell-marker MUC1 while some differentiated into TM cells with CHI3L1 expression. After laser damage, endogenous stem cells, which retained CldU during the chase period, became mitotic and incorporated IdU and expressed Ki67. This study shows exogenous TMSCs have the ability to home to the damaged TM region with potential to repair damaged TM tissue; endogenous stem cells can be activated to divide after damage for potential repairing. This opens a door for exogenous and endogenous stem cell-based therapy for glaucoma.

Poster Schedule

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2	Cynthia Von Zee	FUNCTIONAL COOPERATIVITY BETWEEN CANONICAL AND NON-CANONICAL SIGNALING PATHWAYS IN TGF-β2 MEDIATED ET-1 EXPRESSION
3	Ayman Aljohani	SPHINGOLIPIDS AND CERAMIDES OF HUMAN TRABECULAR MESHWORK
4	Ya Fatou Njie-Mbye	REGULATION OF AQUEOUS HUMOR OUTFLOW BY HYDROGEN SULFIDE
5	Andrew Stothert	NOVEL INHIBITORS OF GLUCOSE-REGULATED PROTEIN 94 (GRP94) REDUCE MUTANT MYOCILIN TOXICITY
6	Mohammad Shahidullah	NONPIGMENTED CILIARY EPITHELIAL CELLS RESPOND TO ACETAZOLAMIDE BY A SOLUBLE ADENYLATE CYCLASE MECHANISM
7	Gillian McLellan	ACTIVE AND LATENT TGF-BETA2 IN THE AQUEOUS HUMOR OF CATS WITH GLAUCOMA
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9	Chris Passaglia	TELEMETRIC SYSTEM FOR INTRAOCULAR PRESSURE MEASUREMENT AND REGULATION IN RATS
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Poster Abstracts

Poster Abstract 1 – MYOCILIN ENHANCES DIFFERENTIATION AND MYELINATION OF OLIGODENDROCYTE THROUGH LINGO-1/Rho A KINASE IN THE CNS

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Oligodendrocytes are the myelin-forming cells of the central nervous system (CNS) and differentiate from proliferative oligodendrocyte precursor cells (OPCs). It has been well established that proteins secreted by astrocytes are essential for migration and differentiation of OPCs. In this study, we report that that myocilin, a known glaucoma associated protein, is produced by optic nerve astrocytes and is involved in differentiation and myelination of OPCs. OPCs were isolated from brain at postnatal 5 days. Treatment of OPCs with myocilin (1μg/ml) for 10 days stimulated the expression of myelin basic protein, myelin associated glycoprotein, and myelin oligodendrocyte glycoprotein as well as process outgrowth. Moreover, myocilin treatment of OPCs and dorsal roll ganglia co-cultures resulted in an increased number of myelin basic protein positive segments on the axons. Secreted myocilin interacted with LINGO-1, a co-receptor of the Nogo A receptor complex. This interaction reduced RhoA-GTPase activity in oligodendrocytes. Inhibition of RhoA-GTPase activity may contribute to the observed stimulation of process outgrowth in OPCs after myocilin treatment. Impaired differentiation of oligodendrocytes was observed in vivo in Myocilin (Myoc) null mice. Optic nerves of these mice exhibited a significant reduction in the thickness of myelin sheath and degeneration of Ranvier nodes compared with wild-type littermates. Optic nerve defects led to delayed latency of visual evoked potential in adult Myoc null mice when compared to wild-type littermates. RNAseq analysis of RNA isolated from the optic nerve of adult wild-type and Myoc null mice demonstrated changes in the levels of several mRNAs encoding proteins involved in myelination. In summary, these results indicate that myocilin is a positive regulator of OPC myelination and differentiation acting through the Lingo-1/RhoA signaling pathway. Therefore, myocilin may be considered as a potential therapeutic target for treatment of demyelination disorders in the CNS including the optic nerve.

Poster Abstract 2 – FUNCTIONAL COOPERATIVITY BETWEEN CANONICAL AND NON-CANONICAL SIGNALING PATHWAYS IN TGF-β2 MEDIATED ET-1 EXPRESSION

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Analysis of aqueous humor (AH) from patients with primary open-angle glaucoma (POAG) reveals marked and potentially pathologic increases in the content of endothelin-1 (ET-1) and transforming growth factor (TGF)-β2. Using cultured primary human trabecular meshwork (TM) cells, we previously demonstrated that TGF-β2 markedly enhances ET-1 synthesis and secretion. Here, we examined the molecular mechanisms by which TGF-β2 mediates ET-1 expression. Primary human TM cells (passages 3-5) were conditioned in serum-free media (x24h) and then incubated in the absence or presence of TGF-β2 (5 ng/ml; x24h). Human TM cells treated with TGF-β2 exhibit a marked (>15-fold) increase in ppET-1 mRNA content compared with vehicle-treated controls, as quantified by qRT-PCR. Similarly, TGF-β2 elicits a marked induction of mature ET-1 peptide secretion, as quantified by ELISA. Specific inhibition of either canonical (TGFβRI/Smad) or non-canonical (Rho GTPase subfamily) signaling pathways completely prevented TGF-β2 mediated increases in ET-1 expression. To determine cooperativity between Smad and Rho GTPase signaling pathways, primary human TM cells were incubated x24h with GGTI-298 (a selective inhibitor of protein geranylgeranylation) prior to TGF-β2 treatment (x30 min). Unexpectedly, TGF-β2 mediated phosphorylation of Smad3 was significantly (40%) reduced in GGTI-298 pretreated TM cells. This suggests for the first time a direct role for Rho GTPase signaling in facilitating TGF-β2 mediated Smad phosphorylation. We conclude that elevated levels of TGF-β2 present in AH of POAG patients may promote aberrant ET-1 synthesis and secretion through activation of a unique Rho GTPase signaling pathway involving canonical Smad phosphorylation. This work was supported by the Department of Veterans Affairs, the Illinois Society for the Prevention of Blindness, the Midwest Eye Banks, and the Richard A. Peritt Charitable Foundation.

Poster Abstract 3 – SPHINGOLIPIDS AND CERAMIDES OF HUMAN TRABECULAR MESHWORK

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To determine the differential profiles of sphingomyelin, sphingoid base, sphingoid base-1-phosphate and ceramide and their quantitative differences between control and glaucomatous trabecular meshwork (TM) derived from human donors. Control and primary open angle glaucoma (POAG) TM samples were collected from cadaver donors. In addition, POAG TM surgical specimens were also procured. All TM samples were collected adhering to tenets of declaration of Helsinki under IRB approved protocols. Lipid extraction was performed using suitable modifications of the Bligh and Dyer method. Protein concentrations were determined using Bradford's method. Lipids were identified and subjected to ratiometric quantification utilizing precursor ion scan (PIS) or neutral ion loss scan (NLS) using appropriate class specific lipid standards on a TSQ quantum Access Max mass spectrometer. We identified several species of sphingomyelin, sphingoid base, sphingoid base-1-phosphate and ceramide to be common between control and glaucomatous TM. A subset of unique sphingolipids and ceramides in some of these classes were also identified in controls but not in glaucoma tissues and vice versa. In conclusion, several sphingolipid and ceramide species were found to be uniquely present in control but absent in the glaucomatous TM and vice versa. Identification of unique lipid species present or absent in the pathophysiological context may contribute insight into glaucoma pathology. This work was partly supported by NIH grants EY016112; EY016112S1; P30-EY14801; F31-EY022872, a Research to prevent blindness (RPB) career award and RPB unrestricted grant to University of Miami. The TSQ Quantum Access Max procurement was supported by Department of Defense Grant W81XWH-09-1-0674. We thank Drs. Tom Mundorf and Edward Rockwood for TM tissues, Dr. Bogdan Gugiu, Dr. Darlene Miller, Maria Carmen Piqueras and Dr. Mike Margolis for their assistance with study design, PCR analyses of Fusarium, bioinformatics and statistical analyses.

Poster Abstract 4 – REGULATION OF AQUEOUS HUMOR OUTFLOW BY HYDROGEN SULFIDE

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There is evidence for the existence of a trans-sulfuration pathway and a pharmacological role of its metabolite, hydrogen sulfide (H_2S) in ocular tissues. In the present study, we tested the hypothesis that H_2S (using sodium hydrosulfide, NaHS as donor, and L-cysteine as substrate) can regulate intraocular pressure (IOP). Methods: The effects of H_2S on aqueous humor outflow facility were measured using a porcine anterior segment perfused organ culture model. Ocular anterior segments explants containing the trabecular meshwork, were mounted on a perfusion chamber and perfused with Dulbecco's Modified Eagle's Culture Medium (DMEM) at constant pressure of 7.35 mmHg maintained at 37°C and 5% CO_2. Once outflow was stable (~ 3 hours), anterior segment explants were administered with different concentrations of L-cysteine (1 nM - 10 μM) and NaHS (100 nM - 10 μM) and outflow was monitored for an additional 4 hours. Vehicle (0.1% saline) was run in parallel. Results: Administration of L-cysteine (1 nM - 10 μM) within 1 hour, caused a dose-dependent increase in outflow facility, reaching a maximal effect at 100 nM [153 ± 7.2% of basal (mean ± SE)]. In addition, NaHS (100 nM - 10 μM) also caused a dose-dependent increase in outflow facility. For instance, 10 μM NaHS caused a significant ($p<0.01$) increase in aqueous humor outflow facility. Interestingly, an equimolar concentration (10 μM) of L-cysteine and NaHS caused a 44% and 25% increase in outflow facility respectively. Conclusion: We conclude that both L-cysteine (as substrate) and NaHS (as donor) can increase aqueous humor outflow in porcine trabecular meshwork, indicating a pharmacological role for H_2S in the regulation of IOP.

Poster Abstract 5 – NOVEL INHIBITORS OF GLUCOSE-REGULATED PROTEIN 94 (GRP94) REDUCE MUTANT MYOCILIN TOXICITY

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Hereditary primary open-angle glaucoma (POAG) can be caused by mutations in the MYOC gene. This genetic form of POAG results from increased intraocular pressure precipitated by the death of a protective cell network called the trabecular meshwork (TM) cells. This cell death is caused by mutations in MYOC, which accounts for ~30% of juvenile-onset open-angle glaucoma cases and a smaller, but substantial percentage of other POAG cases. The MYOC gene encodes for myocilin, a protein secreted from TM cells. Importantly, depletion of myocilin protein does not have adverse effects in the eye. People with nonsense mutations in MYOC that fail to produce any myocilin do not develop glaucoma. Moreover, mice engineered to not express myocilin show no signs of adverse vision or cellular degeneration. Thus, the mutations in MYOC create a toxic gain-of-function by promoting the abnormal amyloidosis of myocilin protein. Based on the features of mutant myocilin and its pathogenicity, we speculated that endoplasmic reticulum (ER) chaperones were heavily involved in its accumulation and potentially, its toxicity. Using targeted knockdown strategies and novel Grp94 inhibitors, we previously determined that Grp94, the ER equivalent of Hsp90, specifically recognizes mutant myocilin, triaging it through ER-associated protein degradation (ERAD). We have now found that most mutant myocilin species are handled the same way; however some are more sensitive than others to Grp94 silencing. Importantly, we now have evidence that silencing Grp94 subverts the toxicity of mutant myocilin accumulation. Based on this we developed new, more potent Grp94 inhibitors that show better potency against mutant myocilin. These inhibitors could regulate myocilin triage and toxicity. Thus targeting Grp94 may be beneficial to those suffering from POAG caused by MYOC mutations.

Poster Abstract 6 – NONPIGMENTED CILIARY EPITHELIAL CELLS RESPOND TO ACETAZOLAMIDE BY A SOLUBLE ADENYLATE CYCLASE MECHANISM

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Soluble adenylyl cyclase (sAC), which is proposed as a cytoplasmic bicarbonate sensor, is highly expressed in the nonpigmented ciliary epithelium (NPE) that plays a key in aqueous humor (AH) secretion. The carbonic anhydrase inhibitor (CAI) acetazolamide (ACTZ), which reduces AH secretion, has been shown to increase cAMP content in certain tissues. Here, we examine the role of sAC in the NPE responses to ACTZ. cAMP was measured by radioimmunoassay in primary cultured porcine NPE. Cytoplasmic pH was measured using BCECF and the rate of pH recovery was measured in cells subjected to acidification by ammonium chloride. A rise in cAMP, detectable within minutes of ATCZ (500 μM) treatment, was prevented by KH7 (50 μM), an sAC inhibitor. ACTZ and methazolamide, a different CAI, both increased the rate of pH recovery and the response was abolished by KH7. Increased pH recovery rate also was observed in cells exposed to IBMX (500 μM). Following 10 min exposure to ATCZ, the abundance of H^+-ATPase (V-ATPase) B1 subunit and sAC was doubled in a plasma membrane-rich fraction, suggesting subcellular translocation. Similar evidence of H^+-ATPase and sAC translocation was observed in NPE exposed to 8-Br-cAMP (1 mM). Bafilomycin A1 (100 nM), an inhibitor of H^+-ATPase, prevented the stimulatory effect of ACTZ on pH recovery. In a parallel study, H^+-ATPase abundance was found to be higher in the plasma membrane of HEK293 cells that overexpress sAC compared to the normal HEK293 cells. HEK cells that overexpress sAC and had high V-ATPase abundance displayed a faster rate of pH recovery and greater sensitivity to KH7. Taken together, the findings suggest ACTZ increases cAMP in a response that involves activation of sAC. Subcellular translocation of H^+-ATPase and an increase in the capacity for proton export in ATCZ treated NPE cells is a cAMP-dependent response. Funding: NIH Grant EY006915.

Poster Abstract 7 – ACTIVE AND LATENT TGF-BETA2 IN THE AQUEOUS HUMOR OF CATS WITH GLAUCOMA

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Transforming growth factor-beta 2 (TGFB) influences the amount and quality of extracellular matrix (ECM) in the eye. Alterations in ECM may impact resistance to aqueous outflow from the anterior chamber, as well as biomechanical properties of the optic nerve head and sclera, contributing to glaucomatous damage. TGFB is normally stored in latent form, in association with several ECM glycoproteins,and signals functions in the eye only when activated. The purpose of this study was to quantify active, latent, and total TGFB in aqueous humor of cats with primary congenital glaucoma (PCG). Concentrations of total and active TGFB were determined by quantitative sandwich ELISA (Quantikine®, R&D Systems, Minneapolis, MN) in aqueous humor samples from 9 PCG (2.54 ±1.26 years) and 9 normal cats (3.22 ± 1.27 years). Latent TGFB concentration was determined as the difference between total and active TGFB in each sample. Concentrations in PCG and normal cats were compared by Mann-Whitney or unpaired t-test. Active TGFB concentrations were significantly increased in cats with PCG (mean ± SD= 153.7 ± 49.26pg/ul,) compared to normal cats (73.3 ± 14.01pg/ul; $p < 0.001$). Median total TGFB concentrations were significantly greater in PCG (3743pg/ul, IQR=2583pg/ul) than in normal cats (2296pg/ul, IQR = 687pg/ul; p=0.001). Mean concentration of latent TGFB was higher in PCG (3620± 979.9pg/ul) than in normal cats (2274pg/ul, p=0.001) and mean ratio of active to total TGFB was slightly greater in PCG (0.04 ± 0.011) than in normal cats (0.03 ± 0.008) but this difference only just statistically significant (p=0.046). In conclusion, concentrations of active, total and latent TGFB are increased in the aqueous humor of glaucomatous cats. Further investigation of the role played by TGFB in glaucoma pathogenesis in this genetic model is planned. Supported by NIH Grants K08 EY018609, T35 OD011078 and P30 EY0016665, and by Research to Prevent Blindness.

Poster Abstract 8 – ANALYSIS OF DISTAL OUTFLOW PATHWAYS OF NORMAL AND PRIMARY OPEN ANGLE GLAUCOMA (POAG) EYES AT LOW AND HIGH PERFUSION PRESSURE

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In POAG eyes, reports have shown that the distal conventional outflow pathway has decreased Schlemm's canal (SC) volume. Histopathologic changes include narrowing and collapse of collector channels (CC), adhesion of SC endothelium to CC orifice walls and herniation/expansion of the juxtacanalicular region resulting in blockage of CC orifices. The purpose of this study was to compare the distal outflow pathway in paired normal (n=3) and glaucoma (n=3) eyes under low (10mmHg) and high (20mmHg) perfusion pressure using three-dimensional micro-computed tomography. SC volume between 10 and 20 mmHg showed a 3.3-fold decrease for normal (0.023±0.18 vs. 0.007±0.004μm^3, $p<0.005$) and a 2.3-fold reduction for POAG (0.007±0.005 vs. 0.003±0.002μm^3, $p<0.006$). Comparison of SC volume between normal and POAG eyes at like pressures showed a significant decrease (10mmHg, 0.023±0.18 vs. 0.007±0.005μm^3, $p<0.015$; 20mmHg, 0.007±0.004 vs. 0.003±0.002μm^3, $p<0.005$). Total number of detectable CCs increased at 20 mmHg in both normal (28.3 vs. 42.7; 10mmHg vs. 20mmHg) and POAG (26.7 vs. 51.3) eyes. Number of occluded CCs also increased as perfusion pressure increased in normal (10 vs. 25 occlusions; 10mmHg vs. 20mmHg) and POAG (3 vs. 71; 10mmHg vs. 20mmHg) eyes. Data suggests an increase in pressure reduces SC volume in normal and POAG eyes and confirms a reduced SC volume between normal and POAG eyes at like pressures. Change in CC numbers with elevated pressure suggests normal and POAG eyes are sensitive to pressure change. Increased number of occlusions in high pressure POAG eyes suggests that narrower SC and reduced number of CCs are available for aqueous outflow and may limit adaptability of POAG eyes to increased pressures. Supported in part by National Institutes of Health research grant EY 21727; Mayo Foundation; and Research to Prevent Blindness, [Lew R. Wasserman Merit Award (MPF) and an unrestricted grant (Department of Ophthalmology, Mayo Clinic)

Poster Abstract 9 – TELEMETRIC SYSTEM FOR INTRAOCULAR PRESSURE MEASUREMENT AND REGULATION IN RATS

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Rats are a popular animal model for glaucoma research. Current methods of inducing ocular hypertension work but are limited by finite success rates, sporadic pressure readings, and variable induction patterns. This research project describes a telemetric system we have developed to monitor and control intraocular pressure levels in rats. The custom designed system consists of a pressure sensor, controller, and pump which are connected to a cannula placed in the anterior chamber of the eye. The system is housed in a box that can be hung on the side of the animal's cage and coupled to the implanted cannula via protective tubing. The results show that the rat eye can be cannulated for several months without causing inflammation or damage to the retina, that the cannula remains patent, and that intraocular pressure remains at normal levels. The results also show that the system can record pressure with a resolution of <0.5 mmHg and hold pressure at a user-prescribed level for days on end. The findings pave the way for our development of an implantable system for glaucoma research in rats and larger animals.

Poster Abstract 10 – INTRAOCULAR PRESSURE (IOP) FOLLOWING UNCOMPLICATED, SMALL-GAUGE PARS PLANA VITRECTOMY (PPV) REPAIR OF PRIMARY RHEGMATOGENOUS RETINAL DETACHMENTS (RD)

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A consecutive interventional case series from one retina practice (n=186) of eyes with or without existing history of primary open-angle glaucoma (POAG) who underwent RD repair with 23 or 25-gauge instrumentation with or without the use of scleral buckle (SB) between 2010 and 2012 was completed. Gas type and concentration (C_3F_8 (perfluoropropane) and SF_6 (sulfur hexafluoride)) used during PPV were documented. IOP was measured by Goldmann applanation during the pre-operative period, on post-operative day 1, week 1, month 1, month 3, and final follow-up day. Mean pre-operative visual acuity 20/200 (logMAR 1.0 +/-1.1) improved to 20/40 (logMAR 0.33 +/-0.43) post-operatively ($p<0.001$). Pre-operative POAG (n=14) was associated with higher IOP at week 1: 23.69 ± 2.02 vs. 16.81 ± 6.43 in eyes without POAG, and final IOP 16.86 ± 2.71 vs. 14.91 ± 3.21 in eyes without POAG ($p<0.01$, $p<0.05$). Mean IOP in eyes with C_3F_8 (n=76) on post-operative day 1 (19.18 ± 9.11) was greater than SF_6 (16.39 ± 8.22) (n=104) (p=0.033) and air (n=6) (11.5 ± 5.86) (p=0.046). In glaucoma eyes, 15-16% C_3F_8 was associated with a higher final IOP than 20-25% SF_6 gas (18.50 ± 2.12 vs. 17.33 ± 3.67) (p=0.047), and cystoid macular edema (CME) was associated with a higher IOP at month 3 than eyes without CME (19.25 ± 3.2 vs. 14.8 ± 2.9) (p=0.027). Use of SB was associated with higher IOP at post-operative day 1 vs. non-SB use (20.88 ± 8.85 vs. 16.94 ± 8.54) (p=0.033). On post-operative day 1, older age had a significant effect on IOP (p=0.0066). IOP elevations are common after gas tamponade for RD repair. C_3F_8, SF_6, and SB-use are associated with higher IOP. Patients with a history of POAG have higher post-operative IOPs and must be closely monitoring in the post-operative period.
COI/Financial Disclosure: None

Poster Abstract 11 – GENE POLYMORPHISM OF PROSTACYCLIN RECEPTOR IN PRIMARY OPEN ANGLE GLAUCOMA

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The purpose of this study was to determine whether gene polymorphisms in the prostacyclin receptor increase the risk of developing glaucoma in the Japanese. A case-control study was carried out on 975 Japanese subjects: 675 primary open-angle glaucoma (POAG) patients including 356 normal-tension glaucoma (NTG) patients; and 300 normal subjects. 2 polymorphisms in prostacyclin receptor gene: PTGIR/Arg227Trp and PTGIR/Arg212Cys (rs4987262) were examined. Of the 2 polymorphisms, PTGIR/Arg212Cys polymorphism had a significantly different distribution in POAG patients; the frequency of the CT+TT genotypes was significantly higher than in controls (P = 0.013; Fisher's exact method). 13 POAG patients had heterozygous (c.747C>T) and no homozygous polymorphism, though controls had neither heterozygous nor homozygous polymorphism. All the cases who had PTGIR/Arg212Cys polymorphism had glaucoma. The minor allele frequency was 1.0%. The possibility of glaucoma caused by prostacyclin receptor gene polymorphism was suggested in the Japanese population.

Poster Abstract 12 – MRP4 AND MRP5 IN THE CILIARY EPITHELIUM AND THEIR POTENTIAL ROLE IN REGULATING EXTRACELLULAR cAMP LEVELS

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Maintenance of normal intraocular pressure (IOP) requires a balance between aqueous humor production by the ciliary epithelium and outflow via the trabecular meshwork. Elevated IOP is associated with the development and progression of glaucoma. The cause of glaucoma is unknown, and requires a better understanding of the mechanisms controlling aqueous humor dynamics. Adenosine and cAMP in aqueous humor are thought to modulate IOP by influencing aqueous humor production and outflow. However, the cellular source and mechanism by which cAMP gets into aqueous humor are unknown. We hypothesize that multidrug-resistance associated proteins 4 (MRP4) and 5 (MRP5) in ciliary epithelium regulate aqueous humor cAMP levels. The purpose was to determine if the ciliary epithelium expresses MRP4 and MRP5, and if their activity can influence extracellular cAMP levels. MRP4 and MRP5 were detected by microarray in human ciliary body from multiple donors. mRNAs (RT-PCR) and protein (immunoblotting) for both transporters were also detected in human ciliary body extracts and in a human ciliary epithelial (CE) cell line. Activity for MRP4/5 in the CE cells was assessed using monochlorobimane-glutathione. The intracellular accumulation of monochlorobimane-glutathione increased dose-dependently in the presence of the MRP inhibitors: indomethacin, probenecid, ibuprofen and furosemide. Isoproterenol (β-adrenergic agonist) and forskolin (adenylyl cyclase activator) each caused a dose- and time-dependent increase in both intra- and extracellular cAMP. Isoproterenol (1μM) and forskolin (1μM) caused a 3-fold (2.7 vs. 8.1 nM) and 8.5-fold (3.0 vs. 25 nM) increase in extracellular cAMP, respectively. The isoproterenol and forskolin stimulation in extracellular cAMP was reduced 50-80% by the MRP inhibitors indomethacin (500 μM) and probenecid (1 mM). We hypothesize that MRP4/5 in ciliary epithelium influences IOP by modulating the cAMP-adenosine signaling pathway in aqueous humor. Funding: NSERC, CIHR and NIH. COI/Financial Disclosure: None

Poster Abstract 13 – THE ROLE OF MFGE-8 IN INTERNALIZATION OF AXONAL DEBRIS IN GLAUCOMA

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Mfge-8 (milk fat globule-EGF factor 8) is an opsonin-like secreted protein that is thought to act as a bridging molecule between apoptotic cells and phagocytes. We show here that this mechanism may play a role not only in the internalization of dying cells, as previously described, but also in a normal axon turnover process in the optic nerve head (ONH) region that may go awry in glaucoma. This hypothesis is based on three sets of data. First, cultured astrocytes show an increase in internalization of purified myelin in the presence of wildtype recombinant Mfge-8, when compared to myelin alone, or myelin incubated with a mutated Mfge-8 unable to bind its integrin receptor. Second, there are increased levels of Mfge-8 protein and mRNA in the ONH of age-related glaucoma model DBA/2J mice, in comparison to the control DBA/2J-Gpnmb^{+} mice. Third, in mice in which intraocular pressure has been increased using a translimbal lasering procedure, lack of Mfge-8 results in increased retinal ganglion cell (RGC) loss, as determined by wholemount retina in situ hybridization with the RGC-specific gamma synuclein mRNA, and increased monocyte infiltration within the optic nerve. Collectively, these data suggest Mfge-8 normally plays a protective role within the optic nerve head, facilitating myelin debris clearance. COI/Financial Disclosure: None

Poster Abstract 14 – MORPHOLOGICAL CHANGES MAY ACCOUNT FOR REDUCED ACTIVE OUTFLOW AREA AND OUTFLOW FACILITY IN EYES WITH PRIMARY OPEN ANGLE GLAUCOMA

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This study aimed to identify structural changes in the aqueous outflow pathway of primary open angle glaucoma (POAG) eyes that may account for reduced active outflow areas and outflow facility (C). Four POAG and four normal human eyes were perfused at 15mmHg to establish a baseline C. The anterior chamber of each eye was exchanged and perfused with a fixed volume of fluorescent microspheres to visualize outflow patterns. All eyes were perfusion fixed. Fluorescent microspheres location in episceral veins and trabecular meshwork (TM) were analyzed globally. All eyes were then dissected into no, low, and high tracer regions. Distribution of microspheres throughout the outflow pathway was analyzed by confocal microscopy in frontal sections. Associated morphological changes for each flow type were further analyzed using both light and electron microscopy. C decreased in POAG eyes compared to normal eyes ($p< 0.05$). Areas of active outflow were segmental in normal eyes and reduced in POAG eyes ($p< 0.05$). Similar morphology, with a discontinuous basement membrane (BM) along the inner wall endothelium of Schlemm's canal (SC), open SC, and open spaces between trabecular beams was seen in high and low tracer regions for both normal and POAG eyes. No tracer regions of normal eyes showed a narrower SC and denser extracellular matrix (ECM) in the TM compared to high tracer regions. No tracer regions of POAG eyes were associated with a collapsed SC, more continuous and thicker BM along the inner wall of SC, and increased ECM beneath the inner wall and in the TM. Morphological differences were consistently found that distinguished active and inactive outflow areas. The morphological changes identified in inactive areas of outflow in POAG may partially explain reduced C and outflow areas and guide us for future identification of specific ECM proteins responsible for these morphological changes in POAG.

Poster Abstract 15 – INHIBITORY ACTION OF HYDROGEN SULFIDE DONOR, ACS 67 ON SYMPATHETIC NEUROTRANSMITTER RELEASE IN ISOLATED BOVINE IRIS-CILIARY BODIES

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There is evidence that hydrogen sulfide (H_2S) regulates sympathetic neurotransmitter release in mammalian anterior uvea (Kulkarni et al., Neurochem Res. 34:400-6, 2009). However, the effect of the H2S donor-latanoprost hybrid, ACS 67 on sympathetic neurotransmission has not been completely elucidated. In the present study, we investigated the effect of, ACS 67 on electrically evoked [^{3}H]NE release in superfused, bovine iris-ciliary bodies (ICB), in vitro. Isolated bovine ICB were incubated in oxygenated Krebs solution containing 2.5μCi/ml of [^{3}H]NE and then prepared for neurotransmitter release using the superfusion method. Release of [^{3}H]NE was elicited by two (S1 and S2) electrical pulses (300 d.c electrical pulses) applied 27 min apart. In the concentration range, 0.1n^M to 30μ^M, ACS 67 and latanoprost (positive control) attenuated field-stimulated [^{3}H]NE release in isolated bovine ICB in a concentration-dependent manner without affecting basal tritium overflow. At an equimolar concentration of 10 μ^M, the rank order of potency was as follows: ACS 67 (37.3%; $p<0.001$) > latanoprost (29.5%; $p<0.001$) on the neurotransmitter release. Although cystathionine β-synthase inhibitor, aminooxyacetic acid (3 m^M) and the ATP-sensitive potassium channel (KATP) inhibitor, glibenclamide (300μ^M) had no effect ($p>0.05$) on [^{3}H]NE release, they both reversed the inhibitory action of ACS 67 (0.1 to 1 μ^M) on the neurotransmitter release. Moreover, the cyclooxygenase enzyme inhibitor, flurbiprofen (3μ^M) reversed the effect of ACS 67 (10μ^M) on field-stimulated [^{3}H]NE release. In conclusion, the in situ release of H_2S, prostanoids and activation of K_{ATP}-channels contribute to the inhibitory action of ACS 67 on sympathetic neurotransmitter release in isolated bovine ICB.

Poster Abstract 16 – NOVEL METHOD FOR DETERMINING RETINAL GANGLION CELL LOSS

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The retinal ganglion cell (RGC) population is the main cell type that is damaged in glaucoma and other optic neuropathies. Current methods quantify RGC loss by calculating cell densities in images of immunostained retina flatmounts. Here we present a method to rapidly measure quantitative differences in mouse retinal ganglion cells using transgenic THY1-CFP mice and a fluorescent microplate reader. Mice expressing cyan fluorescent protein (CFP) driven by the THY1 promoter and control (background) C57BL/6J mice were obtained from Jackson Laboratories. Optic nerve crush was performed unilaterally in each mouse with the fellow eye serving as a control. Two weeks after crush, retinas from THY1-CFP mice were individually homogenized in RIPA buffer with protease inhibitors and homogenates plated into a black 96-well plate. A fluorescent microplate reader (Synergy H1 model, BioTek) was used to measure the average fluorescence per well. Retinal flatmounts of C57BL/6J mice were immunostained for RGC-selective antigens (THY1 or Brn3A) and immunopositive cells counted. Average fluorescence readings (± SEM) for uncrushed and crushed retinas were 49545 ± 1670 and 15175 ± 739 regular fluorescence units. This corresponds to a 69.4% decrease in RGC signal in the eyes that received the optic nerve crush procedure as compared to control eyes. A similar decrease of 62.9% was found in RGC-specific immunopositive cell counts of C57BL/6J flatmounted retinas. Given these comparable outcomes, we conclude that the fluorescent plate-reader protocol provides the same quantitative data as conventional methods but with considerably less time and effort, as imaging and cell counting is avoided. This method could be used as a quick, informative measurement of the progression of RGC loss following optic nerve injury. Supported by USAMRAA DOD W81XWH-12-1-0436, NIH R01 EY14026, NIH P30 EY06360, Research to Prevent Blindness (RPB) and the Katz Foundation. COI/Financial Disclosure: None.

Poster Abstract 17 – GALECTIN-9 INHIBITS STRESS FIBER FORMATION AND PHAGOCYTOSIS IN HUMAN TRABECULAR MESHWORK CELLS

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Cell behavioral changes of human trabecular meshwork (HTM) cells, particularly actin stress fiber formation and phagocytic capacity, have been speculated as causal risk factors for primary open angle glaucoma. In the present study, we investigate the role of galectin-9 (Gal-9), a soluble carbohydrate-binding protein, in regulating these two biological properties of HTM cells. The mRNA levels of Gal-9 in primary HTM cells were determined by quantitative RT-PCR; the protein levels of Gal-9 were assessed by Western blot. Paraffin sections of anterior chamber angle from a normal human eye were immunostained with anti-Gal-9 antibody. Gal-9 was expressed in cultured HTM cells and human TM tissue. Organization of the actin cytoskeleton was assessed in HTM cells treated with exogenous Gal-9. Effect of Gal-9 on phosphorylation of myosin light chain-2 (MLC2) was also examined. Gal-9 inhibited stress fiber formation in HTM cells in dose- and time- dependent manner. Phosphorylation of MLC2 was also reduced in HTM cells treated with Gal-9. Polystyrene latex beads were incubated with HTM cells for 18 hours in the presence or absence of Gal-9 to assess whether changes in phagocytic efficiency were associated with these cytoskeletal changes. Phagocytic capacity of HTM cells was attenuated by exogenous Gal-9 treatment in a dose-dependent manner. Gal-9-mediated attenuation of phagocytic capacity was rescued by addition of lactose, but not sucrose. Viability of HTM cells was not affected by treatment of Gal-9 at 0.1 μM. This study suggests that Gal-9 reduces stress fiber formation in HTM cells through inhibiting phosphorylation of MLC2. In addition, phagocytic capacity of HTM cells is inhibited by exogenous Gal-9 without affecting cell viability. The cytoskeletal changes mediated by exogenous Gal-9 may facilitate the outflow of aqueous humor within the anterior chamber, which may not correlate with the phagocytic capacity of HTM cells.

Poster Abstract 18 – KAPPA OPIOID RECEPTOR ANTAGONIST NOR-BINALTORPHIMINE ATTENUATES ALCOHOL-INDUCED DEATH OF RETINAL GANGLION CELLS

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Previous studies have reported that alcohol causes functional changes in retina by increasing lipid peroxidation product malondialdehyde (MDA) and by decreasing anti-oxidant glutathione (GSH). Yet, their localization in the retina and cell types affected are unclear. Therefore, by feeding Long-Evans rats with alcohol with or without a selective kappa-opioid receptor antagonist, nor-binaltorphimine (nor-BNI), this study performed a systematic immunohistochemical analysis to determine localization of MDA and GSH in the retina, and the cell types involved. Adult Long-Evans rats (6-7 weeks old) were housed and trained to self-administer 15% ethanol (w/v) for 6 weeks. After operant ethanol self-administration stabilized, animals were split into four groups and fed with water, water plus nor-BNI (10 mg/kg), alcohol, and alcohol plus nor-BNI (n=8 rats in each group). Six weeks after treatment, rats were euthanized, their eyes enucleated, and retinal cross retinal cross sections prepared for immunostaining experiments. Retinal cross sections were immunostained with antibodies against GSH, MDA, glial fibrillary acidic protein (GFAP), Iba1, calbindin, acetyltransferase (ChAT), rhodopsin, and conopsin. Retinal flat-mounts were immunostained with antibodies against Brn3a. Apoptotic cell death in the retinas was assessed by TUNEL assays. Compared to the retinas from water-fed rats, GSH levels were reduced and MDA levels were increased significantly in the ganglion cell layer (GCL) of alcohol-fed rats. Reduced levels of GSH and increased levels of MDA correlated with activation of astrocytes and microglial cells, and apoptotic death of RGCs, but not of amacrine cells. Alcohol treatment promoted structural deformities in cone-photoreceptors, but failed to show any effect on rod-photoreceptors. In contrast, alcohol feeding along with nor-BNI decreased reactive gliosis, attenuated microglial activation, and reduced apoptotic death of RGCs. The results presented in this study, for the first time, indicate that kappa-opioid receptor antagonist nor-BNI prevents alcohol-induced apoptotic death of RGCs and offers neuroprotection.

Poster Abstract 19 – THE CALCINEURIN-NFAT PATHWAY REGULATES DEXAMETHASONE (DEX) INDUCED EXPRESSION OF β3 INTEGRIN AND MYOCILIN

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Long-term exposure of trabecular meshwork (TM) cells to DEX causes the upregulation of a number of proteins, such as myocilin, that may play a role in steroid induced glaucoma (SIG). Recent genomic and proteomic studies showed that the expression of αvβ3 integrin is also upregulated by DEX (Clark et al., MCP). αvβ3 integrin signaling is involved in the formation of cross-linked actin networks (CLANs), a structure proposed to play a role in SIG. Western blot, FACS and RT-qPCR analysis confirmed the proteomic studies and showed that the upregulation of αvβ3 integrin was the result of an increase in expression of the β3 integrin subunit as well as an increase in the stability and synthesis of β3 integrin mRNA. A similar increase in the synthesis and translation of myocilin mRNA was also seen. In contrast, DEX did not alter β1 integrin protein or mRNA levels. This increase in β3 integrin expression, like previous studies with myocilin, was found to be a secondary glucocorticoid response as the increase in β3 integrin and myocilin mRNA were not seen until the second day of DEX treatment and were inhibited by cycloheximide. Importantly, the DEX-induced increases in β3 integrin and myocilin mRNA could be inhibited with cyclosporine A, indicating that expression and/or activity was mediated by the calcineurin-NFAT pathway. Interestingly, treatment with DEX for 6 days resulted in the prolonged expression of αvβ3 integrin with an activated conformation that persisted (>10 days) even after removal of DEX. This suggests that signaling events mediated by αvβ3 integrin were also upregulated by DEX. These results suggest that DEX may trigger a long-term dysregulation of αvβ3 integrin signaling in TM cells which could explain the cytoskeleton changes associated with glaucoma. Inhibiting the calcineurin-NFAT pathway with cyclosporine A may be one way to prevent the development of SIG.

Poster Abstract 20 – CiLLIARY EPITHELIAL CELLS INDUCE MAPK SIGNALING IN NORMAL TRABECULAR MESHWORK CELLS CO-CULTURE, A POSSIBLE CONTRIBUTION TO OCULAR TISSUE SIGNALING?

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Cilliary epithelium (CE) includes neuroendocrine activities. The neuropeptides released by the CE to the aqueous humor can serve as messengers to communicate with trabecular meshwork (TM) cells and regulate intraocular pressure. We t hypothesis that TM cells are affected via the aqueous humor by unknown factors released by the CE, resulted in changes in human TM cells morphology, MMP. Co-culture model of human cell lines, nonpigmented ciliary epithelium (ODM), and the HTM cell line were used. HTM cells were cultured with ODM cells for 0.25 0.5, 1, 2, 4 and 8 hr. The effects of ODM cells on HTM cells were examined, focusing on activation of Erk1/2, p38 and the activity of MMPs. Erk1/2 and p38 phosphorylation was measured by Western blot analysis. The activity of MMPs in HTM was determined by zymography. Our result indicates an increase in Erk1/2 and p38 expression up to 30min of co-culture vs. control (NTM-NTM co-culture). MMP-9 activity following co-culture with ODM demonstrated higher activity vs. their match controls. HTM- ODM following intermediate or long incubation time (2,4 & 8hr) showed higher levels of MMP-9 in compare to short co-culture incubation. The HTM-ODM resulted in a time dependent activity elevation of MMP-9 that was measurable as early as 15 minutes and reached a maximum by 8 hr. MMP-2 activity along the experiment did not change significantly. pErk/tErk ratio following ODM-NTM exhibited an decrease at the short incubation time periods vs. NTM-NTM co-culture. At 2hr to 8hr the pErk/tErk ratio was higher for the ODM-NTM.In summary, we demonstrates that TM cells affected by CE, resulted in increased MMP-9 activity and activation of the Erk1/2 pathway. Manipulation of this and related TM signal-transduction pathways may provide targets for developing improved glaucoma treatments.

Poster Abstract 21 – EX VIVO ENGINEERING OF HUMAN LIMBAL EPITHELIAL GRAFTS: THE SUBSTRATE INFLUENCE EXPRESSION OF GENES ASSOCIATED WITH STEMNESS

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Protocols for generation of grafts from human limbal epithelial cells (HLECs) differ to some extent between clinics. Initiation of the cultures is performed using the explant culture technique or a suspension of dissociated cells, and a commonly used substrate is the human amniotic membrane (HAM). Outcome after transplantation is linked to various factors, including to the quality of the generated tissue. In particular, content of cells presenting qualities associated with stemness is of importance. In contrast to HAM, cell culture coated plastic inserts (PIs) provide a readily accessible and standardized substrate. We here expanded HLECs on HAM and on PIs and examined the generated grafts for selected markers associated with stemness and differentiation. Limbal tissues were obtained after removal of the central tissue for transplant purposes. The corneo-limbal ring was divided in samples and placed epithelial side down on either HAM or on PIs in 6 well plates, and cultivated in complex medium (DMEM/F12 supplemented with 5% FBS, DMSO, human EGF, insulin, transferrin, selenium, hydrocortisone, cholera toxin, gentamicin and amphotericin B) for 3 weeks. Samples were processed for analysis using Flow Cytometry and qRT-PCR. After 3 weeks, we have previously reported expression of genes associated with stemness such as ABCG2, P63, OCT4, SOX2, KRT3, and OCLN to be similar in cultures maintained on the two types of substrate. Using FACS sorting, we here detected that expression of P63 was increased in the side population obtained using PIs compared with HAM. In contrast, the expression of ABCG2 was increased in the side population obtained when using HAM as a substrate. In conclusion we show that although genes related to stemness may be similarly expressed in grafts engineered using different substrates, the expression of such genes in the side population obtained after cell sorting may differ considerably.

Poster Abstract 22 – IDENTIFYING CANDIDATE PROTEASES AND THEIR ROLE IN COCHLIN DEGRADATION

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T.A. Carreon, S.K. Bhattacharya Bascom Palmer Eye Institute, University of Miami, Miami, Florida Primary open angle glaucoma (POAG) is a chronic disease most common in people over the age of 50. POAG is ranked as the second leading cause of blindness in the world and is the most common form of glaucoma. This disease is characterized by an increase in intraocular pressure (IOP), which in turn impairs the functionality of the optic nerve resulting in blindness. The specific mechanism that causes the increase in IOP is still unknown. Studies have demonstrated the increased presence of an extracellular matrix (ECM) protein known as cochlin in the glaucomatous trabecular meshwork (TM) in human and mice. In a normal model, cochlin is constitutively produced by TM cells but then regulated through degradation by ECM proteases. In vitro studies have shown that stress, ion concentration changes, and oxidative changes can cause cochlin to multimerize. This multimerized cochlin is believed to play a role in TM remodeling resulting in increased IOP because of the reduced outflow of aqueous humor from the anterior eye. We aim to identify the protease profile of the human TM and decipher the way in which these proteases are affecting cochlin. Preliminary data has identified twenty-six human proteases present in the TM of normal and glaucoma samples. Fourteen of these proteases have been further validated using the PMAP CutBD database. These fourteen proteases have substrates with amino acid sequences present in cochlin itself demonstrating the capability of these proteases to actively splice cochlin. Further work is currently being carried out to determine the exact mechanism in which these proteases act on cochlin in normal as well as diseased samples. This work will contribute to uncovering the exact causative agent of POAG and whether the increase in cochlin is due to lack of degradation or an increase in transcription.

Poster Abstract 23 – PHOSPHOLIPID PROFILES OF CONTROL AND GLAUCOMATOUS HUMAN AQUEOUS HUMOR

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To compare phospholipid (phosphatidylcholine, phosphatidylserine, phosphoethanolamine and phosphatidylinositol) profiles of human control and glaucomatous aqueous humor (AQH). AQH was procured during surgery from POAG and normal patients. Lipid extraction was performed using a modification of the Bligh and Dyer method, protein concentrations were determined using the Bradford method, and for select samples confirmed with Densitometry of PHAST gels. Lipids were identified and subjected to ratiometric quantification using a TSQ Quantum Access Max triple quadrupole mass spectrometer utilizing precursor ion scan (PIS) or neutral ion loss scan (NLS) using appropriate class specific lipid standards. The comparative profiles of phosphatidylcholines, phosphatidylserines, phosphoethanolamines and phosphatidylinositols between control and glaucomatous AQH showed several species common between them. A number of unique lipids in all four phospholipid classes were also identified in control eyes that were absent in glaucomatous eyes and vice versa. To conclude, a number of phospholipids were found to be uniquely present in control, but absent in glaucomatous AQH and vice versa. Compared with a previous study of control and POAG blood, a number of these phospholipids are absent locally in the AQH, as well as systemically. Profiling of lipids will aid in expanding present databases and biological roles that they may play in the anterior segment of the eye. This work was partly supported by NIH grants EY016112, P30-EY14801, a Research to Prevent Blindness (RPB) Career Development Award (to SKB) and a RPB unrestricted grant to University of Miami. The TSQ Quantum Access Max procurement was supported by Department of Defense Grant W81XWH-09-1-0674. We thank Drs. Tom Mundorf and Edward Rockwood for TM tissues, Dr. Bogdan Gugiu for his insightful comments during study design, Yanisis Romero and Juan Carvajal for assistance with mass spectrometry.

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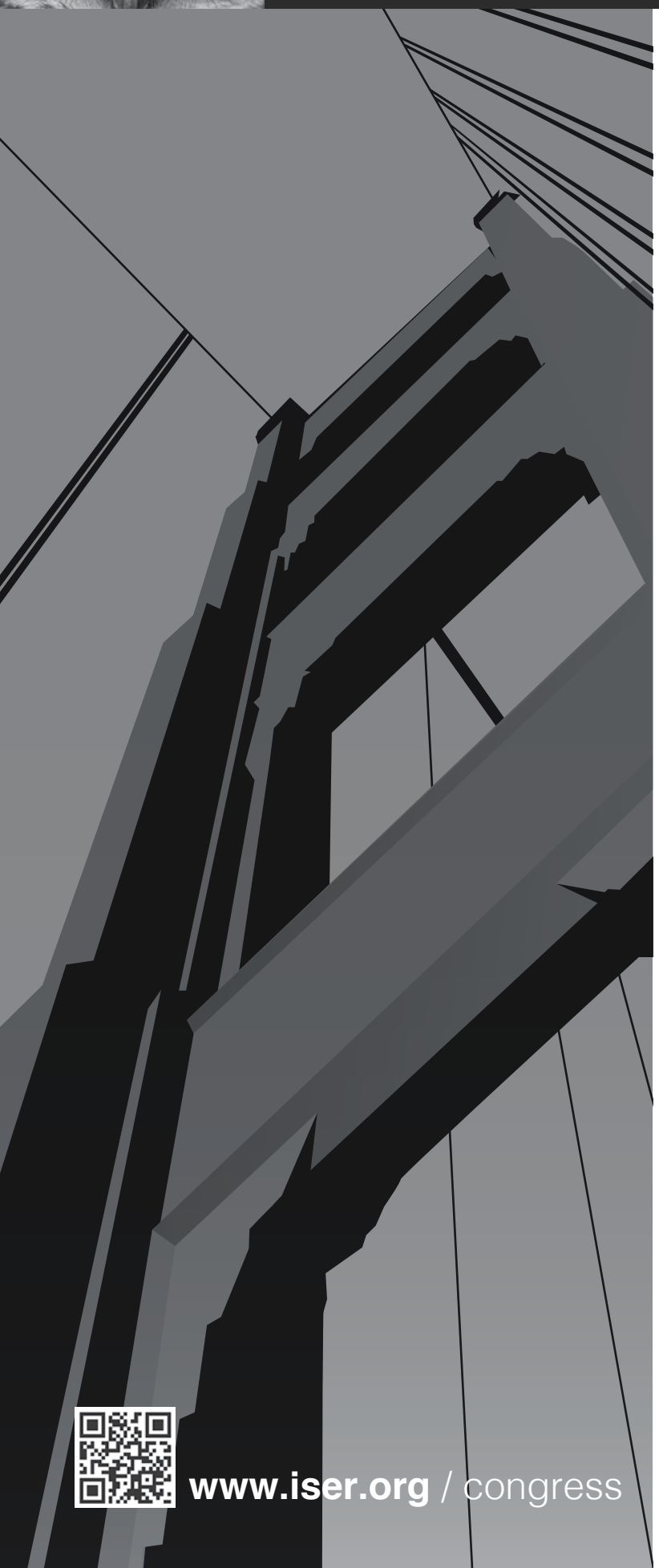

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