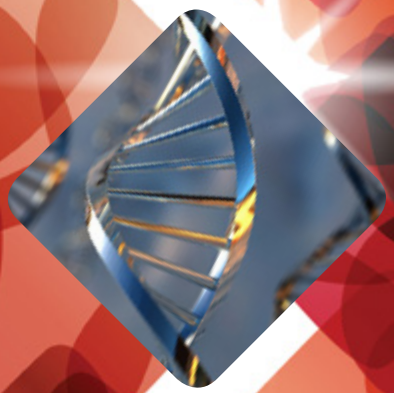
Meeting Program and Abstracts

ASCI / AAP Joint Meeting 2015

April 24 – 26, 2015

The Fairmont Chicago
Chicago, Illinois



APSA
American Physician Scientists Association

www.jointmeeting.org

BOSTON UNIVERSITY

Boston University School of Medicine
Continuing Medical Education

The ASCI / AAP conference is jointly provided by
Boston University School of Medicine and ASCI / AAP.

Special Events at the 2015 ASCI/AAP Joint Meeting

Friday, April 24

ASCI President's Reception
(Invitation only)

6:15 – 7:15 p.m.
Gold Room

ASCI Annual Dinner & New Member Induction Ceremony
(Ticketed guests only)

7:30 – 9:45 p.m.
Moulin Rouge, 1st Floor

Inspiring the Next Generation of Physician-Scientists
Speaker: **Robert J. Lefkowitz, MD**
Duke University

AAP President's Dinner
(Invitation only)

7:00 – 9:00 p.m.
(Off site)

APSA Welcome Reception & Presidential Address

9:00 p.m. – Midnight
John Hancock Center (Off site)

Speaker: **Michael Guo**
APSA President

Saturday, April 25

ASCI Reception for New Members and Young Physician-Scientist Poster Session
(Invited guests and members of the ASCI and AAP only)

6:00 – 8:00 p.m.
Gold Room

Speaker: **Griffin P. Rodgers, MD, MACP**
National Institute of Diabetes, Digestive and Kidney Diseases

AAP Annual Reception & Dinner

7:00 – 10:00 p.m.
Imperial Ballroom

From Lake Wobegon to Stockholm
Speaker: **Peter Agre, MD**
Johns Hopkins University

APSA Dinner

7:30 – 9:00 p.m.
Moulin Rouge

Life as a Physician-Scientist: The Road from Here to There and Everywhere
Speaker: **Liise-anne Pirofski, MD**
Albert Einstein College of Medicine

Dessert Reception
(Open to all attendees)

10:00 p.m. – Midnight
Imperial Foyer

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American Physician Scientists Association

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General Information

Accreditation and Credit Designation

This activity has been planned and implemented in accordance with the Essential Areas and Policies of the Accreditation Council for Continuing Medical Education (ACCME) through the joint providership of the Boston University School of Medicine, the American Society for Clinical Investigation and the Association of American Physicians. Boston University School of Medicine is accredited by the ACCME to provide continuing medical education for physicians.

Boston University School of Medicine designates this live activity for a maximum of 9 *AMA PRA Category 1 Credits™*. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

Nurses and other health professionals will receive a Certificate of Attendance. For information on applicability and acceptance, please consult your professional licensing board.

Course Director

Edward Alexander, MD
Boston University School of Medicine

Program Planning Committee

Committee Members:

Paul Rothman, MD — AAP President
Johns Hopkins University

Stanley Lemon, MD — AAP President-Elect
The University of North Carolina at Chapel Hill

Christine E. Seidman, MD — AAP Vice President
Brigham and Women's Hospital — Harvard Medical School

J. Larry Jameson, MD, PhD — AAP Immediate Past President
University of Pennsylvania Health System, Perelman School of Medicine

Mukesh K. Jain, MD — ASCI President
Case Western Reserve University School of Medicine

Levi Garraway, MD, PhD — ASCI President-Elect
Harvard Medical School, Dana-Farber Cancer Institute

Peter Tontonoz, MD, PhD — ASCI Immediate Past President
University of California, Los Angeles, David Geffen School of Medicine

Lori Ennis — Executive Director AAP,
Ex Officio Committee Member

John Hawley — Executive Director ASCI,
Ex Officio Committee Member

Learning Objectives

As a result of this meeting, participants will be able to:

- Discuss important recent advances in the scientific basis of disease and therapy
- Describe novel strategies to address challenges to the physician-scientist
- Evaluate the roles that improved understanding of these advances and strategies can play in the potential treatment of human disease

Target Audience

Any physician-scientists, trainees, and students, across a broad range of specialties (Basic Research, Cardiology / Cardiovascular Research, Cell and Molecular Biology, Endocrine and Metabolism, Hematology, Immunology, Infectious Diseases, Nephrology, Neurology, Pulmonology, others.

Educational Need

The Joint Meeting Planning Committee strives to represent the cutting edge of biomedical research and medicine. The Committee is especially interested in identifying gaps in knowledge that may exist in the target audience, which consists of physician-scientists, research scientists, clinicians and medical education professionals. The meeting also targets junior scientists and trainees, who benefit from close interaction with senior colleagues.

The 2015 ASCI/AAP Joint Meeting program will feature lectures by accomplished researchers who will discuss state-of-the-art advances in their respective fields. The program is designed to foster in-depth discussions and close interactions among the meeting participants.

DISCLAIMER: THESE MATERIALS AND ALL OTHER MATERIALS PROVIDED IN CONJUNCTION WITH CONTINUING MEDICAL EDUCATION ACTIVITIES ARE INTENDED SOLELY FOR PURPOSES OF SUPPLEMENTING CONTINUING MEDICAL EDUCATION PROGRAMS FOR QUALIFIED HEALTH CARE PROFESSIONALS. ANYONE USING THE MATERIALS ASSUMES FULL RESPONSIBILITY AND ALL RISK FOR THEIR APPROPRIATE USE. TRUSTEES OF BOSTON UNIVERSITY MAKES NO WARRANTIES OR REPRESENTATIONS WHATSOEVER REGARDING THE ACCURACY, COMPLETENESS, CURRENTNESS, NONINFRINGEMENT, MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE OF THE MATERIALS. IN NO EVENT WILL TRUSTEES OF BOSTON UNIVERSITY BE LIABLE TO ANYONE FOR ANY DECISION MADE OR ACTION TAKEN IN RELIANCE ON THE MATERIALS. IN NO EVENT SHOULD THE INFORMATION IN THE MATERIALS BE USED AS A SUBSTITUTE FOR PROFESSIONAL CARE.

General Information

Full Disclosure Policy Affecting CME Activities

Boston University School of Medicine asks all individuals involved in the development and presentation of Continuing Medical Education (CME) activities to disclose all relationships with commercial interests. This information is disclosed to CME activity participants. Boston University School of Medicine has procedures to resolve any apparent conflicts of interest. In addition, faculty members are asked to disclose when any unapproved use of pharmaceuticals and devices is being discussed. It is understood that presentations must give a balanced view of therapeutic options. Faculty use of generic names will contribute to this impartiality. The speaker will make every effort to ensure that data regarding the company's products (or competing products) are objectively selected and presented, with balanced discussion of prevailing information on the product(s) and/or alternative treatments.

2015 Joint Meeting Faculty Disclosures

The following faculty indicated that they have financial relationships to disclose. They have agreed to disclose this to participants. All other named faculty in this program have completed financial disclosure forms and had no financial relationships to report.

First	Last	Disclosure
Richard	Ehman	President, CEO, equity holder, in Resoundant, Inc.
Jeffrey	Friedman	I am listed on the leptin patent and receive a portion of the royalties
Daniel	Geschwind	Consultant, Medical Advisory Board Novartis Consultant Advisory Board SynapDx
Gail	Hecht	Dannon - Scientific Advisory Board member
Rudolf	Jaenisch	Fate Therapeutics - consultant Stemgent - consultant
Jeffrey	Kline	Consultant-- Janssen Pharma Grant support--NIH Consultant--Stago Diagnostica
Mitchell	Lazar	Eli Lilly and Company (Board Member) Pfizer Inc. (Board Member) KDAC Therapeutics, Inc (Board Member) Janssen (Consultant) Merck Inc. (Grant Support)
Jeffrey	Leiden	1. Vertex Pharmaceuticals, Inc. - Chairman, CEO, President & shareholder 2. Quest Diagnostics - Director, shareholder 3. Claurs Ventures - Former Managing Director, Senior Advisor
Stanley	Lemon	Merck - Consultant, research support AbbVie - Consultant, other Theravance - Consultant
Alfred	Sommer	Just finished being a Director of Becton Dickenson. My "retirement" payout will span 10 years
Craig	Thompson	Board of Directors: Merck, Charles River Laboratories, NY Genome Center; SAB/stock holder-Agios; CEO- MSKCC; SAB- MDA, City of Hope
Victor	Velculescu	1. Personal Genome Diagnostics (PGDx) : Founder/ BOD/Officer - Shareholder 2. Quintiles : Member of Sci. Ad. Board - Honorarium

Disclosure of Unlabeled/ Investigational Uses of Drugs or Devices

None of the faculty has indicated that they plan to discuss unlabeled or investigational uses of products or devices.

2015 Joint Meeting Program Committee Disclosures

The following program committee members indicated that they have financial relationships to disclose. They have agreed to disclose this to participants. All other named program committee members in this program have completed financial disclosure forms and had no financial relationships to report.

First	Last	Disclosure
Levi	Garraway	Foundation Medicine: co-founder, stockholder, and consultant (myself) Novartis: sponsored research support and consultant (myself) Boehringer Ingelheim: consultant (myself)
Paul	Rothman	Board Member Cancer Genetics Inc.
Christine	Seidman	CES is a founder and owns shares in Myokardia Inc., a startup company that is developing therapeutics that target the sarcomere.

Claiming CME Credit

Participants who wish to claim CME credit will receive a CME Credit Claim Form and a Required Evaluation with conference materials. In order to claim credit, these forms must be completed and returned to the conference registration desk or mailed to: **Attention Erika Moy, The Kellen Company, 111 Deer Lake Road, Deerfield, IL 60015 no later than May 25, 2015, in order to receive credit.**

General Information

Registration Desk Hours

Friday, April 24	7:00 a.m. – 6:30 p.m.
Saturday, April 25	7:00 a.m. – 5:00 p.m.
Sunday, April 26	7:30 a.m. – 10:00 a.m.

Americans with Disabilities Act

Event staff will be glad to assist you with any special needs (i.e., physical, dietary, etc.). Please contact the Registration Desk at the meeting if you require any special assistance.

ASCI Membership Desk

Visit the ASCI membership desk in the foyer of the International Ballroom for the ASCI's annual report, complimentary copies of JCI Impact, and free JCI beverage mugs.

AAP Membership Desk

Visit the AAP membership desk in the foyer of the International Foyer. The AAP staff will be there to greet you and answer membership questions.

Meeting Evaluation

The ASCI/AAP Joint Meeting Planning Committee needs your input to enhance future meetings. An online meeting evaluation survey will be emailed to you shortly after the Joint Meeting. Your participation in this survey is greatly appreciated.

Poster Session Schedule

Friday, April 24

Poster Setup	1:00 p.m. – 3:00 p.m.
Poster Viewing	6:15 p.m. – 9:30 p.m.

Saturday, April 25

Poster Session with Lunch (presenters must attend)	11:45 a.m. – 1:30 p.m.
Poster Award Discussion Meeting (judges only)	12:45 p.m. – 1:30 p.m.
Poster Dismantle	1:30 p.m. – 2:00 p.m.
Best Poster Awards	3:00 p.m. – 3:15 p.m.

Presenters must be at posters on Saturday, April 25, from 11:45 a.m. – 1:30 p.m. Presenters are not required to be present during all viewing hours.

Best Poster Awards

Best Poster Awards will be given in the amount of $1,000 each. Members of the ASCI, AAP, and APSA will judge posters on scientific novelty, quality and clarity of presentation. **Awards will be presented on Saturday, April 25, from 3:30 – 3:15 p.m.**

Scientific Program

Friday, April 24

Time	Event	Location
7:00 a.m. – 6:30 p.m.	Registration	International Ballroom Foyer
8:30 a.m. – 11:00 a.m.	APSA Business Meeting	Moulin Rouge
11:00 a.m. – 1:00 p.m.	**APSA Session I**	International Ballroom
11:00 a.m. – Noon	 **Jeffrey Kline, MD** *Indiana University* *(sponsored by SAEM)*	
Noon – 12:45 p.m.	 **Gail Hecht, MD** *Loyola University Chicago* *(sponsored by AGA)*	
1:00 p.m. – 3:00 p.m.	Poster Setup	Imperial Ballroom
1:00 p.m. – 3:00 p.m.	**Plenary Session I:** **Understanding Disease Mechanisms to Improve Human Health** Moderators: **Christine Seidman, Mukesh Jain, Dan DelloStritto**	International Ballroom
1:00 p.m. – 1:30 p.m.	 **Michael Welsh, MD** *University of Iowa* **Origins of Cystic Fibrosis Lung Disease***	
1:30 p.m. – 2:00 p.m.	 **Jeffrey Leiden, MD, PhD** *Vertex Pharmaceuticals* **Mutations to Medicines: A Precision Medicine Approach to Cystic Fibrosis**	
2:00 p.m. – 3:00 p.m.	**ASCI and AAP New Member Presentations**	International Ballroom
2:00 p.m. – 2:15 p.m.	 **AAP New Member Presentation** **Katherine L. Nathanson, MD** *University of Pennsylvania* **Testicular Germ Cell Tumors: A Paradigm for Successful GWA Studies in Cancer***	
2:15 p.m. – 2:30 p.m.	 **ASCI New Member Presentation** **Victor E. Velculescu, MD, PhD** *Johns Hopkins University School of Medicine* **Liquid Biopsy Approaches for Detecting and Characterizing Human Cancer***	
2:30 p.m. – 2:45 p.m.	 **AAP New Member Presentation** **Levi Garraway, MD, PhD** *Harvard University* **Principles of Resistance to Targeted Anticancer Agents***	
2:45 p.m. – 3:00 p.m.	 **ASCI New Member Presentation** **Hesham A. Sadek, MD, PhD** *UT Southwestern* **Mechanistic Roadmap to Human Myocardial Regeneration***	
2:00 p.m. – 3:00 p.m.	**APSA Policy Panel** Moderator: **Jennifer Kwan, MD, PhD, PGY2 PSTP** Panelists: **Francis Collins, MD, PhD; Stephen Ostroff, MD; Christopher Burrow, MD; Lloyd Klickstein, MD, PhD**	Moulin Rouge
3:00 p.m. – 3:30 p.m.	Break	International Ballroom Foyer

**Eligible for CME credit*

Scientific Program

Friday, April 24 (continued)

Time	Event	Location
3:30 p.m. – 6:15 p.m.	**Plenary Session II:** **Understanding Disease Mechanisms to Improve Human Health** Moderators: **Paul Rothman, Mukesh Jain, Andrew Harrison**	International Ballroom
3:30 p.m. – 4:00 p.m.	 **Harrington Prize Lecture** **Douglas Lowy, MD** *National Cancer Institute* **Preventing Cervical Cancer and Other HPV-induced Diseases by Vaccination***	
4:00 p.m. – 4:30 p.m.	 **APSA Keynote** **Mitchell Lazar, MD, PhD** *University of Pennsylvania* **Time for Rev-erb Nation***	
4:30 p.m. – 4:45 p.m.	**The Seldin-Smith Legacy**  **Michael S. Brown, MD** *UT Southwestern*  **Warner C. Greene, MD, PhD** *Gladstone Institutes*	
4:45 p.m. – 5:15 p.m.	 **ASCI Presidential Address** **Mukesh K. Jain, MD** *Case Western Reserve University School of Medicine* **Advancing the Mission**	
5:15 p.m. – 5:45 p.m.	 **ASCI Stanley J. Korsmeyer Lecture** **Louis J. Ptáček, MD** *University of California, San Francisco* **Electrical and Episodic Disorders: Channelopathies and Beyond***	
5:45 p.m. – 6:15 p.m.	 **APSA Keynote — Lasker Foundation Award Lecture** **Alfred Sommer, MD, MHS** *Johns Hopkins Bloomberg School of Public Health* **Peregrinations of a Physician Scientist: On Sight and Life***	
6:15 p.m. – 7:15 p.m.	**ASCI President's Reception** (Invitation only)	Gold Room
6:15 p.m. – 9:30 p.m.	Poster Viewing	Imperial Ballroom
7:00 p.m. – 9:00 p.m.	**AAP Offsite President's Dinner** (Invitation only)	Mid-America Club
7:30 p.m. – 9:45 p.m.	 **ASCI Annual Dinner & New Member Induction Ceremony** (Ticketed event) Speaker: **Robert J. Lefkowitz, MD** *Duke University* **Inspiring the Next Generation of Physician-Scientists**	Moulin Rouge
9:00 p.m. – Midnight	 **APSA Welcome Reception & Presidential Address** Speaker: **Michael Guo** *APSA President* Shuttles depart regularly from the Fairmont motor lobby, level B2, beginning at 8:30 p.m.	John Hancock Center

**Eligible for CME credit*

Scientific Program

Saturday, April 25

Time	Event	Location
7:00 a.m. – 5:00 p.m.	Registration	International Ballroom Foyer
7:00 a.m. – 8:00 a.m.	AAP Council Meeting *(AAP Council members only)*	State Room
7:00 a.m. – 8:00 a.m.	Mentoring Breakfast	Moulin Rouge
7:00 a.m. – 8:15 a.m.	Joint Meeting Continental Breakfast	International Ballroom Foyer
7:00 a.m. – 1:30 p.m.	Poster Viewing	Imperial Ballroom
8:15 a.m. – 10:00 a.m.	**Plenary Session III:** **Understanding Disease Mechanisms to Improve Human Health** Moderators: **Linda Fried, Levi Garraway, Stephen Chrzanowski**	International Ballroom
8:15 a.m. – 8:45 a.m.	 **Jeremy Nathans, MD, PhD** *Johns Hopkins University* **New Insights into Vascular Biology in the Eye and Brain***	
8:45 a.m. – 9:15 a.m.	 **Daniel Geschwind, MD, PhD** *David Geffen School of Medicine at UCLA* **Autism: At the Interface of Neuroscience Genetics and Genomic Medicine***	
9:15 a.m. – 9:30 a.m.	**APSA Trainee Presentation** *(see abstract on page 48)* **Ayumi Nakamura, MD** *University of North Carolina at Chapel Hill* **miR-29: A Molecular Timer that Accelerates Aging***	
9:30 a.m. – 10:00 a.m.	 **John A. Kessler, MD** *Northwestern University's Feinberg School of Medicine* **Nanotechnology and Regenerative Neurology***	
10:00 a.m. – 10:30 a.m.	Break	International Ballroom Foyer
10:30 a.m. – 11:45 a.m.	**Plenary Session IV:** **Understanding Disease Mechanisms to Improve Human Health** Moderators: **Paul Rothman, Levi Garraway, Jennifer Kwan**	International Ballroom
10:30 a.m. – 11:00 a.m.	 **Jennifer Doudna, PhD** *University of California, Berkeley* **The Biology and Biotechnology of CRISPRs***	
11:00 a.m. – 11:15 a.m.	**APSA Trainee Presentation** *(see abstract on page 48)* **Jung Park** *University of Iowa* **Tcfap2c Potentiates Tumorigenesis and Cancer Growth in an Activated Neu Model of Mammary Carcinogenesis***	
11:15 a.m. – 11:45 a.m.	 **Rudolf Jaenisch, MD, PhD** *Whitehead Institute, Massachusetts Institute of Technology* **iPS Technology, Gene Editing and Disease Research***	
11:45 a.m. – 1:30 p.m.	Poster Session with Lunch	Imperial Ballroom
12:45 p.m. – 1:30 p.m.	Poster Award Discussion	Royal Room, Level B2
1:30 p.m. – 2:00 p.m.	Poster Dismantle	Imperial Ballroom

**Eligible for CME credit*

Scientific Program

Saturday, April 25 (continued)

Time	Event	Location
1:30 p.m. – 5:45 p.m.	**Plenary Session V:** **Understanding Disease Mechanisms to Improve Human Health** Moderators: **Stanley Lemon, Vivian Cheung, Peter Mittwede**	International Ballroom
1:30 p.m. – 2:00 p.m.	 **APSA Keynote Speaker*** **Craig B. Thompson, MD** *Memorial Sloan Kettering Cancer Center* **Starting at the Bedside: Science in the 21st Century**	
2:00 p.m. – 2:30 p.m.	 **William Crowley, MD** *Massachusetts General Hospital* **Genetic Insights from a Human Disease Model to Define How the Brain Controls Puberty and Reproduction***	
2:30 p.m. – 3:00 p.m.	 **Jeffrey Friedman, MD, PhD** *Rockefeller University* **Leptin and the Neuronal Control of Glucose Metabolism***	
3:00 p.m. – 3:15 p.m.	Best Poster Awards	
3:15 p.m. – 3:40 p.m.	Break	International Ballroom Foyer
3:40 p.m. – 4:00 p.m.	AAP Business Meeting	International Ballroom
4:00 p.m. – 4:30 p.m.	 **AAP Presidential Address** **Paul Rothman, MD** *Johns Hopkins University School of Medicine* **Medicine in 2055**	
4:30 p.m. – 5:00 p.m.	 **Kober Lecturer** **Philip Fred Sparling, MD** *University of North Carolina School of Medicine* **A Doctor's Dilemma: Choices Amidst Change***	
5:00 p.m. – 5:45 p.m.	**Kober Medal Presentation**  Recipient: **Francis Collins, MD, PhD** *National Institutes of Health*  Presenter: **David Ginsburg, MD** *University of Michigan Medical School*	
6:00 p.m. – 7:00 p.m.	**APSA Panel: Women in Medicine and Science** Panelists: **Louise Laurent, MD, PhD; Claire Pomeroy, MD, MBA; Hilary Reno, MD, PHD; Hanna Stevens, MD, PhD**	Crystal Room
6:00 p.m. – 8:00 p.m.	 **ASCI Reception for New Members and Young Physician-Scientist Poster Session** (Invited guests and members of the ASCI and AAP only) Speaker: **Griffin P. Rodgers, MD, MACP** *National Institute of Diabetes, Digestive and Kidney Diseases*	Gold Room

**Eligible for CME credit*

Scientific Program

Saturday, April 25 (continued)

Time	Event	Location
7:00 p.m. – 10:00 p.m.	 **AAP Reception and Dinner** Speaker: **Peter Agre, MD** *Johns Hopkins University* **From Lake Wobegon to Stockholm**	Imperial Ballroom
7:30 p.m. – 9:00 p.m.	 **APSA Dinner** (Ticketed event) Speaker: **Liise-anne Pirofski, MD** *Albert Einstein College of Medicine* *(sponsored by IDSA)* **Life as a Physician-Scientist: The Road from Here to There and Everywhere**	Moulin Rouge
10:00 p.m. – Midnight	Dessert Reception (open to all attendees)	Imperial Ballroom Foyer

Sunday, April 26

Time	Event	Location
7:30 a.m. – 10:00 a.m.	Registration	International Ballroom Foyer
8:00 a.m. – Noon	**APSA Program**	Moulin Rouge
8:00 a.m. – 9:30 a.m.	Interest Groups & Mentorship	Moulin Rouge
9:00 a.m. – 10:00 a.m.	ASCI/AAP Joint Council Wrap-up Meeting *(Invited guests only)*	State Room
9:30 a.m. – 10:00 a.m.	 **APSA Keynote** **Richard L. Ehman, MD** *Mayo Clinic* *(sponsored by RSNA)* **Patents as Proxies: Should Inventions Be Used as an Outcome Metric in Medical Research?***	Gold Room
10:00 a.m. – 11:00 a.m.	**APSA Panel:** **Technological Innovation in Biomedical and Translational Research**	Gold Room
10:00 a.m. – 11:00 a.m.	**APSA Panel:** **Ethical Implications and Use of Big Data**	Crystal Room
11:00 a.m. – Noon	**APSA Panel:** **Post-Graduate Opportunities**	Gold Room
11:00 a.m. – Noon	**APSA Panel:** **MSTP Admissions**	Crystal Room
Noon – 2:00 p.m.	**APSA Residency Luncheon**	Moulin Rouge

**Eligible for CME credit*

Speaker Biographies

(listed in alphabetical order by last name)

Peter Agre, MD

Dr. Peter Agre is currently the Bloomberg Distinguished Professor at the Johns Hopkins School of Medicine and Bloomberg School of Public Health. He shared the 2003 Nobel Prize in Chemistry for discovering the aquaporins, a family of water channel proteins found throughout nature and underlying numerous physiological processes and clinical disorders.

Dr. Agre is also deeply involved in multiple global issues. He currently directs the Johns Hopkins Malaria Research Institute, leading field research in Zambia and Zimbabwe. As chair of the Committee on Human Rights of the National Academies, he led efforts on behalf of imprisoned scientists, engineers, and health professionals worldwide. Past President of the American Association for the Advancement of Sciences, he leads scientific diplomatic visits and meetings with leaders of countries including Cuba, DPRK (North Korea), Myanmar (Burma), and Iran.

Dr. Agre graduated from Augsburg College and Johns Hopkins School of Medicine.

Michael S. Brown, MD

Dr. Michael Brown received a BA in chemistry in 1962 and an MD degree in 1966 from the University of Pennsylvania. He was an intern and resident at the Massachusetts General Hospital, and a postdoctoral fellow with Dr. Earl Stadtman at the National Institutes of Health. In 1971, he came to UT Southwestern where he rose through the ranks to become a professor in 1976. He is currently Paul J. Thomas Professor of Molecular Genetics and Director of the Jonsson Center for Molecular Genetics at UT Southwestern.

Dr. Brown and his long-time colleague, Dr. Joseph L. Goldstein, together discovered the low density lipoprotein (LDL) receptor, which controls the level of cholesterol in blood and in cells. They showed that mutations in this receptor cause Familial Hypercholesterolemia, a disorder that leads to premature heart attacks in one out of every 500 people in most populations. They have received many awards for this work, including the U.S. National Medal of Science and the Nobel Prize for Medicine or Physiology.

Francis Collins, MD, PhD

Dr. Francis S. Collins is the Director of the National Institutes of Health (NIH). In that role he oversees the work of the largest supporter of biomedical research in the world, spanning the spectrum from basic to clinical research.

Dr. Collins is a physician-geneticist noted for his landmark discoveries of disease genes and his leadership of the international Human Genome Project, which culminated in April 2003 with the completion of a finished sequence of the human DNA instruction book. He served as director of the National Human Genome Research Institute at the NIH from 1993–2008.

Before coming to the NIH, Dr. Collins was a Howard Hughes Medical Institute investigator at the University of Michigan. He is an elected member of the Institute of Medicine and the National Academy of Sciences, was awarded the Presidential Medal of Freedom in November 2007, and received the National Medal of Science in 2009.

William Crowley, MD

Dr. William Crowley is the Daniel Podolsky Professor of Medicine at Harvard Medical School and the Massachusetts General Hospital. His research has focused on defining the physiology, pathophysiology, and genetic control of the hypothalamic GnRH neurons that determine the onset of sexual maturation at puberty and control adult reproduction.

He pioneered the use of GnRH agonists in 1978 to desensitize the GnRH receptor and induce a 'biochemical castration' to treat children with sexual precocity. Once he defined this biology of GnRHa, their wider use to suppress testosterone levels in men with prostate cancer and estrogen in women with endometriosis and breast cancer quickly followed. He developed the use of pulsatile GnRH to induce a normal puberty in men and women whose Isolated GnRH deficiency had caused them to fail to undergo a normal puberty.

Over the past 15 years, Dr. Crowley and his colleagues have studied patients with IGD to identify several new genes/ signaling pathways that control the human reproduction such as Kisspeptin, Prokineticin 2, and FGF8/FGFR1.

Dr. Crowley received The Fred Conrad Koch Award, The Endocrine Society's highest scientific award; the Ipsen International Juried Prize in Endocrinology; and the Clinical Investigator Awards of both the NIH and The Endocrine Society. He also served as the founding Director of the Clinical Research Program at the MGH the national Clinical Research Forum group for 16 years.

Jennifer Doudna, PhD

Dr. Jennifer A. Doudna is a professor of biochemistry and molecular biology at UC Berkeley. Her research seeks to understand how non-coding RNA molecules control the expression of genetic information.

Dr. Doudna holds a PhD in biological chemistry from Harvard University.

After serving as a member of the Yale University faculty for eight years, during which time she was promoted to Henry Ford II Professor of Molecular Biophysics and Biochemistry, she joined the UC Berkeley faculty in 2002.

She has been a Howard Hughes Medical Institute investigator since 1997 and a member of the National Academy of Sciences since 2002. She was named to the American Academy of Arts and Sciences in 2003 and elected to the Institute of Medicine in 2010. She is a recipient of the Lurie Prize from the Foundation for the NIH, and a co-recipient of the Paul Janssen Award in Biomedical Science.

Dr. Doudna has served as a consultant to pharmaceutical companies including Gilead and Merck, and is on the Scientific Advisory Board of biotechnology companies including eFFECTOR Therapeutics and Caribou Biosciences.

Speaker Biographies

Richard L. Ehman, MD

Dr. Richard L. Ehman is Professor of Radiology at the Mayo Clinic and an Emeritus member of the Mayo Clinic Board of Trustees. His research program is focused on developing new imaging technologies. He holds more than 40 patents and many of these inventions have been commercialized and are widely used in medical care.

He has served as chair of the Radiology and Nuclear Medicine Study Section of the National Institutes of Health ((NIH), as a member of the Advisory Council of the National Institute of Biomedical Imaging and Bioengineering of the NIH, and as a member of the Council of Councils of the NIH. Dr. Ehman was awarded the Gold Medal of the International Society for Magnetic Resonance in Medicine in 1995 for his research contributions and the Outstanding Researcher Award of the Radiological Society of North America in 2006. He was elected to the Institute of Medicine of the National Academies of Science in 2010.

Dr. Ehman served as president of the International Society for Magnetic Resonance in Medicine in 2002–2003. He serves on the Board of Directors of the Radiological Society of North America (RSNA) and will be president in 2017. He was president of the Academy of Radiology Research from 2012 to 2014. Dr. Ehman is currently the president of the Society for Body Computed Tomography and Magnetic Resonance.

Jeffrey Friedman, MD, PhD

Dr. Jeffrey Friedman studies the molecular mechanisms that regulate food intake and body weight. Genetic studies in mice led to the identification of leptin, a hormone made by fat tissue that plays a key role in regulating weight. Current studies explore the mechanisms by which leptin controls feeding behavior and body weight. Studies to identify other key regulators are also under way.

The recent identification of the hypothalamic cells that express the leptin receptor is enabling Dr. Friedman and his colleagues to delineate the precise neuronal effects of leptin and the mechanisms by which this single molecule can alter a complex behavior.

He graduated from Rensselaer Polytechnic Institute and, in 1977 at the age of 22, received his MD from Albany Medical College of Union University. After completing two residencies at Albany Medical Center Hospital, he came to Rockefeller as a postgraduate fellow and associate physician in 1980. In 1986 he received his PhD. He was named head of laboratory in 1991 and Marilyn M. Simpson Professor in 1999. He has been an investigator at the Howard Hughes Medical Institute since 1986.

A member of the National Academy of Sciences and its Institute of Medicine, his honors include the King Faisal International Prize in Medicine, the BBVA Frontiers of Knowledge Award in Biomedicine, the Fondation IPSEN Endocrine Regulation Prize, the Albert Lasker Award for Basic Medical Research, the Shaw Prize in Life Science and Medicine, the Keio Medical Science Prize, the Jessie Stevenson Kovalenko Medal, the Danone International Prize for Nutrition, the Gairdner Foundation International Award and the Passano Foundation Award.

Levi Garraway, MD, PhD

Dr. Levi Garraway is an Associate Professor of Medicine in the Department of Medical Oncology at the Dana-Farber Cancer Institute, Harvard Medical School, and a Senior Associate Member of the Broad Institute. He is the inaugural Director of the Joint Center for Cancer Precision Medicine at the Dana-Farber, Brigham and Women's Hospital, and the Broad Institute, and co-Director of the Cancer Genetics Program at the Dana-Farber/Harvard Cancer Center.

Dr. Garraway received his AB in Biochemical Sciences from Harvard College in 1990, and his MD and PhD degrees from Harvard Medical School in 1999. Thereafter, he completed his internship and residency in Internal Medicine at the Massachusetts General Hospital, where he also served as Medical Chief Resident in 2003. He received fellowship training in Medical Oncology at the Dana-Farber Cancer Institute.

Dr. Garraway has made seminal research contributions in cancer genomics, drug resistance, and precision (or "personalized") cancer medicine. He published the first whole genome sequencing studies of prostate cancer, and has led major cancer genomics initiatives in both melanoma and prostate cancer. He was also the first to describe ways in which an important subtype of melanoma becomes resistant to several new targeted therapies.

Dr. Garraway is perhaps best known for his contributions to precision cancer medicine. He described the first high-throughput adaptation of genomic technology to profile human tumors for hundreds of cancer gene mutations that could guide therapeutic choices. This research has guided precision medicine initiatives at many cancer centers worldwide. It also inspired the launch of Foundation Medicine, Inc., a genomics-based cancer diagnostics company co-founded by Dr. Garraway. In September of 2013, Foundation Medicine completed an initial public offering that raised $122 million.

In 2009, Dr. Garraway was inducted into the American Society for Clinical Investigation and is currently President-Elect of that organization. Dr. Garraway has been the recipient of several awards and honors, including the Partners in Excellence Award, the prestigious New Innovator Award from the National Institutes of Health, and the Block Award for Outstanding Cancer Research from the Ohio State University. In 2013, Dr. Garraway received the Paul Marks Prize, awarded every other year to three top cancer scientists age 45 or younger. In 2014, Dr. Garraway was awarded the Jane Cooke Wright Lectureship by the American Association for Cancer Research. This award recognizes an outstanding scientist who has made meritorious contributions to the field of cancer research and who has, through leadership or by example, furthered the advancement of minority investigators in cancer research.

Daniel Geschwind, MD, PhD

Dr. Daniel Geschwind is the Gordon and Virginia MacDonald Distinguished Professor of neurology, psychiatry and human genetics at the UCLA School of Medicine. He is director of the Neurogenetics Program and the Center for Autism Research and Treatment (CART) and co-director of the Center for

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Neurobehavioral Genetics in the Semel Institute at UCLA.

Dr. Geschwind obtained an AB in psychology and chemistry at Dartmouth College and his MD/PhD at Yale School of Medicine prior to completing his internship, residency (Neurology), and postdoctoral fellowship at UCLA. He joined the UCLA faculty in 1997, founding the neurogenetics program. His laboratory aims to develop a mechanistic understanding of neuropsychiatric diseases, such as autism and neurodegenerative diseases, and their relationship to the range of normal human higher cognitive function and behavior. By using computational and bioinformatic methods in addition to wet laboratory experimentation, the lab's goal is to help develop effective therapeutics for neurologic and psychiatric disorders.

Dr. Geschwind is also a strong advocate for large-scale data and biomaterial sharing, and has provided scientific oversight for the Autism Genetic Resource Exchange (AGRE). He has published more than 300 papers and serves on the editorial boards of several journals including *Biological Psychiatry, Cell, Human Molecular Genetics, Neurobiology of Disease, Neuron* and *Science.*

He has received numerous awards including the Scientific Service Award from Autism Speaks in 2007, the Ruane Prize for Child and Adolescent Psychiatric Research from the Brain and Behavior Foundation in 2012, the Taking on Tomorrow Innovation Award (Research/Scientific Breakthrough in Autism)—Boston Children's Hospital in 2013 and is an elected member of the Institute of Medicine of the National Academies.

David Ginsburg, MD

Dr. David Ginsburg is James V. Neel Distinguished University Professor of Internal Medicine, Human Genetics, and Pediatrics at the University of Michigan and a Howard Hughes Medical Institute Investigator.

He received his BA from Yale, MD from Duke, and postdoctoral clinical and research training at the Brigham and Women's Hospital and Children's Hospital in Boston. The Ginsburg laboratory studies the components of the blood-clotting system and how disturbances in their function lead to human bleeding and thrombotic disorders.

Ginsburg is a member of the ASCI, AAP, IOM, American Academy of Arts and Sciences, and National Academy of Sciences. He is recipient of the ASCI Stanley J. Korsmeyer Award, E. Donnall Thomas Prize, the AHA Distinguished Scientist Award, and the AAMC Award for Distinguished Research in the Biomedical Sciences. He is a past president of the ASCI and has served on the Councils for the NAS and IOM.

Warner C. Greene, MD, PhD

Dr. Warner C. Greene is Director and Nick and Sue Hellmann Distinguished Professor, Gladstone Institute of Virology and Immunology; Professor of Medicine, Microbiology and Immunology at the University of California, San Francisco (UCSF), and co-director of the UCSF-Gladstone Center for AIDS Research. In 2007 he became President of the Accordia Global Health Foundation, whose first center of excellence, the Infectious Diseases Institute at Makerere University in Kampala, Uganda, has already trained more than 6,700 health care workers from 27 countries, is caring for 30,000 HIV-infected patients, and has outreach programs improving the health of nearly 500,000 people in remote rural Uganda. Since 2013 he has guided Accordia as Executive Chairman.

He received his BA degree from Stanford University and MD and PhD degrees from Washington University School of Medicine, followed by internship and residency training in Internal Medicine at Massachusetts General Hospital. Previous positions include Senior Investigator at the National Cancer Institute, Professor of Medicine at Duke University Medical Center, and HHMI Investigator.

Dr. Greene's memberships include the American Academy of Arts and Sciences and the Institute of Medicine, and he is a fellow of the American Academy for the Advancement of Science. He is also past President of the Association of American Physicians. In his career he has mentored more than 120 students and fellows and is the author of more than 360 scientific papers.

Gail Hecht, MD

Dr. Gail Hecht earned a MS in Medical Microbiology at the University of MO-Columbia followed by an MD from Loyola University Stritch School of Medicine. She completed her residency in Internal Medicine at the University of Minnesota-Minneapolis and received Fellowship training in Gastroenterology at Brigham and Women's Hospital, Harvard Medical School, in Boston.

Most of her career was spent at the University of Illinois-Chicago where she rose to the rank of Professor of Medicine and Microbiology/Immunology and Chief of Digestive Diseases and Nutrition. She was recruited to Loyola University Chicago in 2013 where she is currently Professor of Medicine and Microbiology/Immunology, Chief, Gastroenterology and Nutrition, and Assistant Dean for Medical Student Research.

The focus of Dr. Hecht's research is host-pathogen interactions with specific emphasis on enteropathogenic *Escherichia coli*. Her goal is to determine how the bacterial effector proteins that are translocated into intestinal epithelial cells by these pathogens alter host cell physiology including tight junction barrier function, active ion transport processes, and the innate immune response. Her research is funded by both the NIH and the VA. She also has an interest in the gut microbiota and its impact on intestinal function and health.

She serves as Editor-in-Chief of a new journal *Gut Microbes* published by Taylor & Francis. Dr. Hecht has been very active in the American Gastroenterological Association functioning as Chair of the Intestinal Disorders Section of the AGA Council, Basic Research Councilor to the Governing Board and ultimately serving as President from 2009-2010, only the second woman to serve in that capacity.

Speaker Biographies

Rudolf Jaenisch, MD, PhD

Dr. Rudolf Jaenisch is professor of biology at M.I.T. and a founding member of the Whitehead Institute for Biomedical Science. His work focuses on understanding epigenetic regulation of gene expression, which has led to major advances in our understanding of embryonic stem cells and "induced pluripotent stem" (IPS) cells. In addition to its stem cell work, Jaenisch's lab is investigating epigenetic mechanisms for certain types of cancer and for brain development, studying how conditions such as Rett Syndrome occur.

Jaenisch received his doctorate in medicine from the University of Munich in 1967. Before coming to Whitehead, he was head of the Department of Tumor Virology at the Heinrich Pette Institute at the University of Hamburg.

He has coauthored more than 375 research papers, was appointed to the National Academy of Sciences in 2003 and has received numerous prizes and recognitions including the Robert Koch Prize for Excellence in Scientific Achievement (2002), the Wolf Prize in Medicine (2011), the President's National Medal of Science, 2010 (awarded in 2011), and the Otto Warburg Medal (2014) from the German Society for Biochemistry and Molecular Biology. He currently serves as the president of the International Society for Stem Cell Research.

Mukesh K. Jain, MD

Dr. Mukesh Jain is the Ellery Sedgwick Jr. Chair & Distinguished Scientist; Director, Case Cardiovascular Research Institute and Professor of Medicine, Case Western Reserve University School of Medicine; and Scientific Director, Harrington Discovery Institute and Chief Research Officer, Harrington Heart & Vascular Institute at University Hospitals Case Medical Center.

Dr. Jain is recognized for discovery of essential roles for the transcription factor family Krüppel-like factors (KLFs) in inflammation and metabolism. In addition, he has translated this corpus of work into animals and humans, thus implicating KLFs in the physiology of inflammatory and metabolic diseases including sepsis, myopathy, diabetes and vascular dysfunction. As a direct result of this work, KLFs are now increasingly viewed, together with classic regulators such as NFkB and nuclear receptors, as nodal determinants of cellular inflammation and metabolism.

Dr. Jain's clinical and academic contributions are recognized by numerous awards and honors including election to the American Society for Clinical Investigation, Association of American Physicians, and the Association of University Cardiologists. Dr. Jain is a member of the American Heart Association's Advancement of Science and Basic Science Council, serves on multiple editorial boards, and has been the recipient of numerous NIH grants.

Dr. Jain received his MD from the University of Buffalo School of Medicine. He completed his residency in internal medicine at the Beth Israel Hospital in Boston and completed a cardiovascular medicine fellowship at Brigham & Women's Hospital, Harvard Medical School.

John A. Kessler, MD

Dr. John A. Kessler is the Davee Professor of Stem Cell Biology at Northwestern University's Feinberg School of Medicine, Director of the Feinberg Neuroscience Institute, and Director of the Northwestern University Stem Cell Institute. He was chairman of the Davee Department of Neurology for 12 years and is an active clinician as well as a stem cell biologist.

Dr. Kessler is currently the principal investigator on three NIH grants in the field of stem cell research and is part of an NIH Biophysical Research Partnership focused on use of nanotechnology for repair of the damaged nervous system. He is also the principal investigator in a multicenter gene therapy trial for the treatment of neuropathy. He is the founding Editor-in-Chief of the *Annals of Clinical and Translational Neurology,* chairman of a scientific advisory panel for the March of Dimes, and is on the Board of Trustees of two hospitals. He is the author of more than 275 scientific publications.

Dr. Kessler has won numerous awards including a Peabody Award for his documentary film, "Terra Incognita: The Perils and Promise of Stem Cell Research." He earned his AB degree from Princeton University and his MD from Cornell University Medical College. He has been a member of the AAP since 2005.

Jeffrey Kline, MD

Dr. Jeffrey Kline received his MD from the Medical College of Virginia, and then did an emergency medicine residency followed by a research fellowship at the Carolinas Medical Center. He now serves as Vice Chair of Research in emergency medicine and a professor of physiology.

Dr. Kline studies blood clots, the people they affect, and the providers who care for those people. His diagnostic research interests focus on human affect analysis, pretest probability and novel breath-based instruments to reduce medical imaging. His human treatment research includes randomized trials of fibrinolysis and inhaled nitric oxide to reduce heart damage from blood clots in the lungs. He derived and validated a decision rule to help emergency physicians reduce unnecessary diagnostic tests for low-risk patients with symptoms of blood clots in the lungs.

His current work focuses on using the human face as a diagnostic instrument to further help doctors make smart decisions about diagnostic testing for blood clots. His laboratory work focuses on mechanisms and treatment of acute pulmonary hypertension from pulmonary embolism, animal models of pulmonary embolism, and a nanoparticle-delivered enzyme, plasmin to promote clot lysis without increasing risk.

He helped set up an advanced treatment program to treat patients with severe PE in the hospital, and he also created and runs a clinic specifically to allow patients diagnosed with blood clots in the emergency department to treat themselves at home, rather than in the hospital.

Speaker Biographies

Mitch Lazar, MD, PhD

Dr. Mitchell Lazar is the Sylvan Eisman Professor of Medicine and Genetics, the Chief of the Division of Endocrinology, Diabetes, and Metabolism, and the Director of the Institute for Diabetes, Obesity, and Metabolism at the University of Pennsylvania. He received his undergraduate degree in Chemistry from the Massachusetts Institute of Technology, then received a PhD in Neurosciences and an MD from Stanford University. He trained in Internal Medicine at Brigham and Women's Hospital and in Endocrinology at the Massachusetts General Hospital before joining the University of Pennsylvania faculty in 1989.

Dr. Lazar discovered the circadian nuclear receptor Rev-erba, as well as its heme ligand, transcriptional repression, and interactions with corepressors and histone deacetylases. His work has demonstrated the fundamental importance of Rev-erb and its corepressor complex in the physiology of circadian rhythms and organismal metabolism. Dr. Lazar also discovered that another nuclear receptor, PPARγ, is predominantly expressed in adipocytes and also pioneered the linkage of PPARγ to adipocyte differentiation, insulin resistance, and type 2 diabetes. He also led the way to a genome-wide understanding of PPARg function, and discovered resistin as a novel adipocyte hormone that impairs insulin action and as the first member of a previously unknown family of secreted resistin-like molecules.

He has given named lectures throughout the world, and has served as a member of the Board of Scientific Councilors of the NIDDK as well as many editorial and scientific advisory boards. He has been elected to the American Society for Clinical Investigation and the Association of American Physicians, and received two NIH Merit Awards, the Van Meter Award of the American Thyroid Association, the BMS Freedom to Discover Award, the Richard Weitzman Award, Edwin B. Astwood Lecture Award and Gerald D. Aurbach Lecture Award from The Endocrine Society, and the Stanley Korsmeyer Award of the American Society for Clinical Investigation. He was elected to the Institute of Medicine of the U.S. National Academy of Sciences in 2006, and to the American Academy of Arts and Sciences in 2008.

Robert J. Lefkowitz, MD

Dr. Robert Lefkowitz is James B. Duke Professor of Medicine and Professor of Biochemistry at the Duke University Medical Center. He has been an Investigator of the Howard Hughes Medical Institute since 1976.

Dr. Lefkowitz began his research career in the late 1960s and early 1970s, when there was no clear consensus that receptors as conceived of by pharmacologists even existed. His group spent 15 years developing techniques for radioligand binding, solubilization, purification, and reconstitution of the four adrenergic receptors known at the time.

In 1986, Dr. Lefkowitz transformed the understanding of what had become known as G protein coupled receptors (GPCRs), when he and his colleagues cloned the gene and cDNA for the β_2 adrenergic receptor, and recognized its sequence homology with rhodopsin, thus establishing them as the first members of a new family of proteins, the Seven Transmembrane Receptors (7TMRs). This superfamily is now known to be the largest, most diverse, and most therapeutically accessible.

Since then, Dr. Lefkowitz has continued to revolutionize the GPCR field through the cloning of eight adrenergic receptor subtypes and the first serotonin (5HT1A) receptor; discovery and cloning of the G protein coupled receptor kinases (GRKs) and β-arrestins; and discovery of "biased" signaling through β-arrestins or G proteins. Most recently, he has been applying the tools of structural biology to understand biased signaling at atomic level resolution.

He has received numerous awards and honors, including the National Medal of Science, the Shaw Prize, the Albany Prize, and the 2012 Nobel Prize in Chemistry. He was elected to the USA National Academy of Sciences in 1988, the Institute of Medicine in 1994, and the American Academy of Arts and Sciences in 1988.

Jeffrey Leiden, MD, PhD

Dr. Jeffrey Leiden is the Chairman, President and Chief Executive Officer of Vertex Pharmaceuticals, and has served as a member of Vertex's board of directors since 2009. Dr. Leiden has more than 20 years of scientific, commercial and financial experience in the pharmaceutical and biotechnology industries as well as clinical and scientific experience in academia as a practicing cardiologist and molecular biologist.

Prior to joining Vertex, Dr. Leiden was a Managing Director for Clarus Ventures, a life sciences venture capital firm he joined in 2006. From 2000 to 2006, he served as President and Chief Operating Officer, and Chief Scientific Officer at Abbott Laboratories where he ran Abbott's global pharmaceuticals business. While at Abbott, he helped launch multiple breakthrough medicines, including Humira for rheumatoid arthritis and other autoimmune diseases and Kaletra for HIV infection, among others.

He is a fellow of the American Academy of Arts and Sciences, and an elected member of the Institute of Medicine of the National Academy of Sciences. He is currently a trustee of the Brigham and Women's Hospital and a member of the Scientific Advisory Board of Boston Children's Hospital. Dr. Leiden earned his BA, MD, and PhD degrees from the University of Chicago.

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Douglas Lowy, MD

The 2015 Harrington Prize recognizes Dr. Douglas Lowy's key discoveries that led to development of the human papillomavirus (HPV) vaccine to prevent cancer. The vaccine developed by Dr. Lowy (in collaboration with Merck and GlaxoSmithKline), and approved by the FDA in 2006, was the first licensed vaccine to prevent cancer by guarding against the sexually transmitted infection that causes the disease. It is estimated that the HPV vaccine can afford close to 100% protection and thus Dr. Lowy's research has the potential to prevent virtually all of the many cancers caused by HPV.

Dr. Lowy received his MD from New York University School of Medicine. Between 1970 and 1973, he was a research associate in the Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, NIH. He trained in internal medicine at Stanford University and dermatology at Yale University, and started his laboratory at the NCI in 1975.

In addition to his own research, Dr. Lowy is a leader in promoting public health issues related to HPV-associated diseases, especially cervical cancer in developing nations. He is an effective advocate for sustainable comprehensive cervical cancer control in the developing world.

He is an elected member to the prestigious National Academy of Sciences and is recipient of numerous awards and honors including the National Medal of Technology and Innovation from President Obama in 2014.

Jeremy Nathans, MD, PhD

Dr. Jeremy Nathans is a professor in the Departments of Molecular Biology and Genetics, Neuroscience, and Ophthalmology at the Johns Hopkins Medical School and an Investigator of the Howard Hughes Medical Institute. He earned bachelor's degrees in Life Sciences and Chemistry from M.I.T. (1979), and a PhD in Biochemistry (1985) and an MD (1987) from Stanford Medical School. After one year of postdoctoral research at Genentech, he joined the faculty at the Johns Hopkins Medical School and the Howard Hughes Medical Institute in 1988.

Dr. Nathans is best known for his fundamental discoveries in basic and clinical vision research. Current research in the Nathans laboratory focuses on pattern formation in embryonic development, the pathogenesis of retinal disease, and vascular biology and disease, including regulation of the blood-brain-barrier and blood-retinal-barrier.

Dr. Nathans' research and teaching have been recognized with numerous awards, including the Newcomb-Cleveland Prize from the American Association for the Advancement of Science, the Initiatives in Research Award of the National Academy of Sciences, the Champalimaud Award in Vision Research, the Scolnick Prize in Neuroscience from M.I.T., the Lifetime Achievement Award in Biomedical Science from Stanford Medical School, and the Golden Apple Award from the American Medical Student Association. He is an elected member of the National Academy of Sciences and the Institute of Medicine.

Katherine L. Nathanson, MD

Dr. Katherine L. Nathanson is an Associate Professor in the Division of Translational Medicine and Human Genetics in the Department of Medicine at the Perelman School of Medicine of the University of Pennsylvania. She also serves as co-leader of the Cancer Control Program and Chief Oncogenomics Physician for the Abramson Cancer Center, as well as Director of Genetics for the Basser Center for BRCA Research. Dr. Nathanson received her BA from Haverford College, and her MD from the University of Pennsylvania School of Medicine. She completed two residencies, one in Internal Medicine at the Beth Israel Hospital, Boston and the other in Clinical Genetics at the Children's Hospital of Philadelphia and the Hospital of the University of Pennsylvania, while also completing her post-doctoral fellowship in cancer genetics in the laboratory of Dr. Barbara Weber.

Dr. Nathanson both runs a research laboratory and has a busy clinical practice. Clinically, she manages patients with inherited cancer susceptibility syndromes, in particular those associated with renal and neuroendocrine tumors. Her laboratory focuses on the elucidation of the inherited and somatic genetics of cancer, specifically in testicular germ cell tumors (TGCT), pheochromocytomas/paragangliomas (PCC/PGL), melanoma, and familial breast cancer. Dr. Nathanson is the leader of the TCGT post-GWAS international consortium and has published extensively on the susceptibility genetics of TGCT. She also has multiple publications in the area of somatic genetics of melanoma, examining genetic and genomic predictors of response and resistance to targeted therapies.

She has a long term interested in hereditary breast cancer, and has multiple on-going studies studying both BRCA1/2 mutation carriers, examining mutation specific risk and doing sequencing of BRCA1/2 mutation associated tumors, characterizing moderate penetrance genes and identifying novel genes in high risk women without BRCA1/2 mutations. Dr. Nathanson also studies the somatic genetics of PCC/PGL, and is the co-leader of the Analysis Working Group (AWG) for the PCC/PGL TCGA, as well as a member of the AWG for TGCT.

Liise-anne Pirofski, MD, FIDSA

Dr. Liise-anne Pirofski is professor of medicine, microbiology, and immunology, the Selma and Jacques Mitrani Professor of Biomedical Research at the Albert Einstein College of Medicine, and chief of the Division of Infectious Diseases at the Albert Einstein College of Medicine and Montefiore Medical Center in Bronx, New York.

She earned her BA at the University of California, Berkeley, and her MD from Albert Einstein College of Medicine, trained in internal medicine at Bellevue Hospital and New York University Medical Center, as well as in infectious diseases followed by post-doctoral training at Albert Einstein College of Medicine and Montefiore Medical Center. She has had a NIH-funded research program on immunity to encapsulated pathogens for more than two decades, served extensively on NIH study sections and external advisory panels on infectious diseases, and is an editor of *mBio* and *Infection and Immunity.*

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She is also a co-developer of the Damage-response framework, an integrated theory of microbial pathogenesis and the pathogenesis of infectious diseases. Dr. Pirofski is a member of the Association of American Physicians and the American Academy of Microbiology and a fellow of IDSA and the American College of Physicians. She has received the Harry Eagle Award for Outstanding Basic Science Teaching and a Faculty Mentoring Award from Einstein and is the 2015 recipient of the ASM William A. Hinton Research Training Award.

Louis J. Ptáček, MD

Dr. Louis Ptáček is the 2015 recipient of the American Society for Clinical Investigation's Stanley J. Korsmeyer Award for research leading to the development of the field of ion channel defects, known commonly as channelopathies.

Recently, Dr. Ptáček and collaborators were the first to identify genes in humans responsible for regulating the circadian rhythm and sleep systems. This work has opened avenues to improved understanding of the similarity between the internal clocks of humans and other organisms, and has allowed investigation into the potential relationship between circadian system pathologies and those present in channelopathies.

Dr. Ptáček received his MD from the University of Wisconsin, Madison, in 1986, and completed his residency in neurology at the University of Utah in 1990. Afterward, he performed postdoctoral studies in human genetics at the University of Utah. Dr. Ptáček currently holds the John C. Coleman Distinguished Professorship in the Department of Neurology at the University of California, San Francisco, where he is also Director of the Neurogenetics Program. He has been a Howard Hughes Medical Institute Investigator since 1997.

Dr. Ptáček was elected to the ASCI in 2000, to the Institute of Medicine in 2007, to the Association of American Physicians in 2009, and to the National Academy of Sciences in 2012.

Griffin P. Rodgers, MD, MACP

Dr. Griffin Rodgers was named Director of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)—one of the National Institutes of Health (NIH)—on April 1, 2007. He had served as NIDDK's Acting Director since March 2006 and had been the Institute's Deputy Director since January 2001. As the Director of NIDDK, Dr. Rodgers provides scientific leadership and manages a staff of over 600 employees and a budget of ~$2.0 billion.

Dr. Rodgers received his undergraduate, graduate and medical degrees from Brown University in Providence, RI. He performed his residency and chief residency in internal medicine at Barnes Hospital and the John Cochran VA, respectively, at Washington University in St. Louis, MO. His fellowship training in hematology was in a joint program of the NIH with George Washington University and the Washington Veterans Administration Medical Center. In addition to his medical and research training, he earned an MBA, with a focus on the business of medicine/science, from Johns Hopkins University in 2005.

As a research investigator, Dr. Rodgers is widely recognized for his contributions to the development of the first effective—and now FDA-approved—therapy for sickle cell anemia. He was a principal investigator in clinical trials to develop therapy for patients with sickle cell disease and also performed basic research that focused on understanding the molecular basis of how certain drugs induce gamma-globin gene expression. In addition, he and his collaborators have reported on a modified blood stem cell transplant regimen that is highly effective in reversing sickle cell disease in adults and is associated with relatively low toxicity. He has been honored for his research with numerous awards including the 1998 Richard and Hinda Rosenthal Foundation Award, the 2000 Arthur S. Flemming Award, the Legacy of Leadership Award in 2002 and a Mastership from the American College of Physicians in 2005, among others.

Dr. Rodgers has been an invited professor at medical schools and hospitals in the U.S. and internationally. He has been honored with many named lectureships and commencement speeches at American medical centers and has published over 200 original research articles, reviews, and book chapters, has edited four books and monographs, and holds 3 patents.

Paul Rothman, MD

Dr. Paul B. Rothman is the Frances Watt Baker, MD, and Lenox D. Baker Jr., MD, Dean of the Medical Faculty, vice president for medicine of The Johns Hopkins University, and CEO of Johns Hopkins Medicine. As dean/CEO, Rothman oversees both the School of Medicine and the Johns Hopkins Health System, which together encompass six hospitals, hundreds of faculty and community physicians and a self-funded health plan.

A molecular immunologist, Rothman's research focused on immune system molecules known as cytokines. Specifically, he investigated the role these molecules play in the normal development of blood cells, as well as the abnormal blood-cell development that leads to leukemia. He also studied the function of cytokines in immune system responses to asthma and allergies. His work was consistently funded by the National Institutes of Health.

Rothman's honors include a James S. McDonnell Foundation Career Development Award, a Pfizer Scholars Award, a Pew Scholar in the Biomedical Sciences Award, a Leukemia Society of America Scholar Award and the Pharmacia Allergy Research Foundation International Award. He is a member of the American Society for Clinical Investigation and is a Fellow of the American College of Physicians. He was elected as a Fellow of the American Association for the Advancement of Sciences and as a member of the American Clinical and Climatological Association. He is serving as president of the Association of American Physicians for 2014–15.

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Hesham Sadek, MD, PhD

Dr. Hesham A. Sadek is currently an Assistant Professor of cardiology at UT Southwestern Medical Center. He is a practicing cardiologist with board certification in Internal Medicine, Cardiology and Echocardiography. Dr. Sadek's research focuses on mammalian heart regeneration, as well as the link between metabolism and cell cycle regulation.

He was born in Manchester, England, obtained his medical degree from Ain Sham University in Egypt, and his PhD from Case Western Reserve University in Cleveland, Ohio. He completed clinical training in internal medicine and cardiology at the University Hospitals of Cleveland, and a post doctoral-fellowship in stem cell biology at UT Southwestern Medical Center.

Dr. Sadek's independent laboratory is funded by grants from the National Institute of Health, the American Heart Association and NASA. He is the an author on numerous original publications, including senior author publications in *Science, Nature, Cell, Cell Stem Cell, PNAS, JACC* and *Blood* among others. Dr. Sadek is the recipient of several national and international awards including the American College of Cardiology Douglas Zipes Distinguished Young Scientist Award.

Alfred Sommer, MD, MHS

Dr. Sommer is a Gilman Scholar and University Distinguished Service Professor at Johns Hopkins University; Johns Hopkins Professor of Epidemiology, Ophthalmology, and International Health; and Dean Emeritus of the Johns Hopkins Bloomberg School of Public Health. His current research interests include child survival and blindness prevention strategies, micronutrient interventions, and the interface between public health and clinical medicine.

He received his MD from Harvard Medical School (1967) and his Master of Health Science in Epidemiology from the Johns Hopkins School of Public Health (1973). He has published six books and more than 300 scientific articles, and has received numerous awards including the *Albert Lasker Award for Clinical Research*, the *Warren Alpert Foundation Prize*, and the *Duke Elder International Gold Medal for Contributions to Ophthalmology.* He has delivered more than 40 named lectureships, including the Jackson Memorial Lecture (American Academy of Ophthalmology), Duke Elder Oration (Royal College of Ophthalmologists), De Schweinitz Lecture (College of Physicians, Philadelphia), and Doyne Lecture (Oxford Ophthalmologic Congress), among others.

Dr. Sommer is a member of both the U.S. National Academy of Sciences and the Institute of Medicine. Sommer has received the Laureate Award of the American Academy of Ophthalmology, and was elected to the ASCRS "Ophthalmology Hall of Fame."

Philip Fred Sparling, MD

Dr. Philip Sparling was on the faculty in medicine and microbiology and Immunology at the University of North Carolina Chapel Hill from 1969 until he retired in August 2014, where he served as division chief of infectious diseases, chair of microbiology and immunology, and then chair of medicine. Prior to that he served at the Centers for Disease Control in Atlanta from 1964–1966 in the Venereal Disease Research Laboratories, and as a postdoctoral fellow in microbiology under Bernard Davis at Harvard from 1966–1968.

His work focused for many years on the genetics of antibiotic resistance and the molecular pathogenesis of gonococcal disease. In later years he also served as director of the SE Regional Center of Excellence in Biodefense and Emerging Infections (2005–2014), and also directed a long standing Center for Sexually Transmitted Infections Research.

He graduated from Princeton University (1958) and Harvard Medical School (1962). He received graduate medical training in Internal medicine at Massachusetts General Hospital (MGH) from 1962–1964 and at MGH in infectious diseases (1968–1969).

He served as President of the Infectious Diseases Society of America from 1996–1997; was a charter member of the Forum on Emerging Infections of the IOM; was a consultant to industry and foundations; and was active in service to the NIH. He worked as a clinician and teacher throughout, and continues to do so in retirement. He and his wife Joyce also traveled to and paddled canoes down some of the great rivers of Arctic Canada, where they befriended many of the local Inuit.

Craig B. Thompson, MD

Dr. Craig B. Thompson is the President and Chief Executive Officer of Memorial Sloan Kettering Cancer Center (MSKCC). Dr. Thompson is a board-certified internist and medical oncologist with extensive research experience in cancer, immunology and translational medicine. His current research focuses on the regulation of cellular metabolism during cell growth/differentiation and on the role that metabolic changes play in the origin and progression of cancer.

Dr. Thompson is a member of the Institute of Medicine, the National Academy of Sciences, the American Academy of Arts and Sciences, and the Medical Advisory Board of the Howard Hughes Medical Institute.

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Victor E. Velculescu, MD, PhD

Dr. Victor Velculescu is co-director of cancer biology and professor of oncology and pathology at the Johns Hopkins University Kimmel Cancer Center. He is internationally known for his genomic discoveries in human cancer.

Dr. Velculescu developed SAGE (serial analysis of gene expression) and used this method to perform the first transcriptome analysis in eukaryotic cells. Subsequently, he developed digital karyotyping (DK) for analysis of structural genomic alterations and together with his colleagues performed the first sequence analysis of the coding genome in human cancers, including breast, colorectal, brain, pancreatic, and ovarian cancers. These analyses identified a variety of genes not previously known to be involved in neoplasia, including PIK3CA gene as one of the most highly mutated genes in human cancer.

More recently, his group has developed PARE (personalized analysis of rearranged ends) for non-invasive liquid biopsy approaches for tumor detection and monitoring. These discoveries provide insights into the mechanistic features and pathways underlying human cancer and provide new opportunities for individualized diagnostic and therapeutic approaches.

Michael Welsh, MD

Dr. Michael Welsh earned his MD from the University of Iowa, completed his internship and residency in Internal Medicine there, and trained in pulmonary medicine and research at the University of California—San Francisco and the University of Texas in Houston. He then joined the University of Iowa where he is a Professor of Internal Medicine and Molecular Physiology and Biophysics. He directs the Cystic Fibrosis Research Center and Pappajohn Biomedical Institute. He is an Investigator of the Howard Hughes Medical Institute.

Dr. Welsh's clinical activities focus on pulmonary diseases. His research has concentrated on understanding the biology and pathogenesis of cystic fibrosis and on the development of new treatments. He is also investigating the biology of protons and acid-sensing ion channels involved in normal and pathological neuronal function.

Dr. Welsh served as president of the American Society for Clinical Investigation and as president of the Association of American Physicians. He has been awarded the Cecile Lehman Mayer Research Award, American College of Chest Physicians; the Doris F. Tulcin Cystic Fibrosis Research Award; the Paul di Sant'Agnese Distinguished Scientific Achievement Award; the J. Burns Amberson Award, American Thoracic Society; the Regents Award for Faculty Excellence; and the Second Annual Distinguished Mentoring Award, UI Carver College of Medicine. He has been elected to the Institute of Medicine, the American Academy of Arts and Sciences, and the National Academy of Sciences.

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2015 ASCI Young Physician-Scientist Awards

The ASCI is pleased to recognize the recipients of its 2015 Young Physician-Scientist Awards. This program recognizes young physician-scientists who are early in their first faculty appointments and who have made notable achievements in their research. Awardees present their work at the ASCI Reception for New Members and Young Physician-Scientist Poster Session, on Saturday, April 25, 6 to 8 p.m.

Omar Abdel-Wahab, MD (Poster: YPSA-1)
Memorial Sloan Kettering Cancer Center

Oren Becher, MD (Poster: YPSA-2)
Duke University

Eugenio Cingolani, MD (Poster: YPSA-3)
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Oscar Colegio, MD, PhD (Poster: YPSA-4)
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Cincinnati Children's Hospital Medical Center

J. Travis Hinson, MD (Poster: YPSA-7)
Brigham and Women's Hospital — Harvard Medical School

Rajan Jain, MD (Poster: YPSA-8)
Perelman School of Medicine at the University of Pennsylvania

Peng Ji, MD, PhD (Poster: YPSA-9)
Northwestern University

J. Michelle Kahlenberg, MD, PhD (Poster: YPSA-10)
University of Michigan Health System

Alex Kentsis, MD, PhD (Poster: YPSA-11)
Memorial Sloan Kettering Cancer Center

Jeffery Klco, MD, PhD (Poster: YPSA-12)
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University of Michigan Health System

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Washington University School of Medicine

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Massachusetts General Hospital

Hao Zhu, MD (Poster: YPSA-40)
Children's Research Institute at UT Southwestern

YPSA Poster Abstracts

YPSA-1

SRSF2 Mutations Contribute to Myelodysplasia Through Mutant-Specific Effects on Exon Recognition

Eunhee Kim[1], Janine O. Ilagan[2,3], Yang Liang[4], Gerrit M. Daubner[5], Stanley Lee[1], Yorgo Modis[6,7], Frederic H.-T. Allain[5], Stephanie Halene[4], Robert K. Bradley[2,3], and *Omar Abdel-Wahab*[1,8]

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Mutations within genes encoding spliceosomal proteins are the most common class of mutations in patients with myelodysplastic syndromes, yet it is currently not well understood how these mutations impact hematopoiesis or RNA splicing. Here we studied mutations in the spliceosomal protein SRSF2 which is mutated in 20% of MDS and 50% of chronic myelomonocytic leukemia (CMML) patients. In order to understand the functional impact of *SRSF2* mutations on hematopoiesis and splicing, we generated isogenic human and murine systems to study the effect of expression of commonly occurring mutation in *SRSF2* compared with isogenic hematopoietic cells with loss of SRSF2. Using conditional knockin mice expressing the common point mutation in *Srsf2* (Srsf2P95H) from its endogenous locus compared with conditional deletion of 1 or 2 copies of *Srsf2*, we identified that mutations affecting SRSF2 directly impair hematopoietic differentiation *in vivo*, which is not due to SRSF2 loss of function. Transcriptomic analysis of human and murine *SRSF2* mutant cells identified that SRSF2 mutations alter SRSF2's preference for specific exonic splicing enhancer (ESE) motifs. Analysis of wildtype and mutant SRSF2 peptides in *in vitro* cell-free RNA binding assays likewise identified that mutations in *SRSF2* impair its binding, avidity, and preference for specific ESE sequences normally recognized by wildtype SRSF2. Integration of murine and human transcriptomic data identified recurrent missplicing of numerous key hematopoietic regulators in the presence of mutant SRSF2. This includes *SRSF2* mutation-dependent splicing of *EZH2* that triggers nonsense-mediated decay, which, in turn, results in impaired hematopoietic differentiation. These data provide a mechanistic link between a mutant spliceosomal protein, alterations in splicing of key regulators, and impaired hematopoiesis.

YPSA-2

Modeling Diffuse Intrinsic Pontine Glioma in Mice: ACVR1 Mutations, H3.1 K27M Mutations, and p53 Loss

Christine M. Hoeman[1,2], Francisco J. Cordero[2], Kyle G. Halvorson[1,2,3,4], David L. Corcoran[5], Javad Nazarian[6], and *Oren J. Becher*[1,2,3]

[1]Division of Hematology-Oncology, Department of Pediatrics; Duke University Medical Center; Durham, NC, USA; [2]Department of Pathology; Duke University Medical Center; Durham, NC, USA; [3]Preston Robert Tisch Brain Tumor Center; Duke University Medical Center; Durham, NC, USA; [4]Division of Neurosurgery, Department of Surgery; Duke University; Durham, NC, USA; [5]Institute for Genome Sciences and Policy; Duke University; Durham, NC, USA; [6]Integrative Systems Biology; George Washington University; Washington, DC, USA

Diffuse intrinsic pontine gliomas (DIPGs) are the leading cause of death for children with brain tumors. Recently we and others have discovered activating mutations in ACVR1, which encodes for Activin A receptor type I (ALK-2), a bone morphogenetic protein (BMP) receptor, in approximately 20% of DIPG patients. Furthermore, DIPGs with mutant ACVR1 also commonly harbor H3.1 K27M mutations. However, whether these mutations can promote tumor formation in vivo remains to be demonstrated. Herein we report that ACVR1 mutations are capable of inducing glioma-like lesions; however, the gain of function H3.1 K27M mutation and p53 loss are required for lesion formation. Additionally, lesions display a mesenchymal phenotype as evident by Stat3 activation, high CD44 expression and minimal Olig2 immunostaining. These results are intriguing, as BMP signaling has primarily been shown to be a tumor suppressor pathway in gliomagenesis, but in this context it is a novel pathway implicated in promoting mesenchymal gliomagenesis.

YPSA-3

Biological Pacemaker Created by Cocal Tbx18 Gene Transfer in a Pre-clinical Model of Complete Heart Block

***Eugenio Cingolani*, Yu-Feng Hu, James Dawkins, Hee Cheol Cho, Eduardo Marbán**

Cedars-Sinai Heart Institute, Los Angeles, CA, USA

We have recently demonstrated that re-expression of an embryonic transcription factor, TBX18, leads to conversion of ordinary cardiomyocytes to pacemaker cells of the sinoatrial node (SAN). These induced SAN pacemaker cells (iSANs) are driven by membrane- and Ca2+-clock mechanisms of automaticity, and adopt the long & lean cell morphology of native pacemaker cells. Gene- and cell-based biological pacemakers (BioP) have been demonstrated successfully in animal models of heart block. We sought to investigate if the iSANs could create biological pacemaker (BioP) activity in vivo in a pre-clinical, porcine model of complete heart block. Adenoviral vectors expressing either TBX18 or GFP were delivered into the right ventricular septum via venous catheters after radiofrequency ablation of the atrioventricular node. Animals were followed for 14 days by implanted telemetry and serial pacemaker interrogations. Physical activity was measured by a built-in accelerometer in the implanted telemetry.

YPSA Poster Abstracts

Electro-anatomic activation maps and electrophysiological studies were conducted 2 weeks after the gene delivery to test BioP function and potential inducible arrhythmias. BioP activity was seen in Tbx18-transduced animals starting at day 2 and persisted during follow-up. Mean heart rate (HR) at day 7 was significantly higher in Tbx18-transduced animals compared to GFP controls (HR=79.6±2.2 vs. 64.5±3.8bpm, $p<0.05$). Moreover, higher daytime HR (89.0±6.6 vs. 69.8±5.5bpm, $p<0.05$), HR during maximal activity (91.2±7.5 vs. 69.9±5.6bpm, $p<0.05$), and the increment of 11.2% in mean daily activity were seen in the TBX18-transduced group. Earliest BioP activation site by electro-anatomic mapping were compatible with pacing map of the injection site. No significant differences in QT dispersion were seen. Additionally, no inducible arrhythmias were seen after programmed stimulation in both groups. TBX18-mediated iSANs create BioP activity in a clinically-relevant porcine model of complete heart block. The minimally invasive delivery method combined with local/systemic safety profiles brings this approach closer to clinical adaptation.

YPSA-4

Tumor-Derived Lactic Acid Functionally Polarizes Tumor-Associated Macrophages

Oscar R. Colegio **and Ruslan Medzhitov**
Departments of Dermatology and Immunobiology, Yale University, New Haven, CT, USA

Macrophages play critical roles in the maintenance of tissue homeostasis. To perform these functions, macrophages must have the capacity to assess the functional states of their client cells, typically the parenchymal cells in the various tissues in which macrophages reside. Tumors exhibit many features of abnormally developed tissues and organs, including cellular composition and tissue architecture. Similarly to macrophages in normal tissues and organs, tumor-associated macrophages perform key homeostatic functions that allow for tumor maintenance and growth. However, the signals involved in communication between neoplastic cells and macrophages are poorly defined. We determined that lactic acid produced by neoplastic cells, as a by-product of aerobic or anaerobic glycolysis, has a critical function in signaling, by inducing the expression of vascular endothelial growth factor and the M2-like polarization of tumor-associated macrophages. Furthermore, we found that this effect of lactic acid is mediated by the transcription factor, hypoxia-inducible factor 1α (HIF1α). Finally, we determined that the lactic acid-induced expression of arginase by macrophages has a critical role in tumor growth. Collectively, these findings define a mechanism of communication between macrophages and their client cells, including neoplastic cells. This communication most likely evolved to promote homeostasis in normal tissues but can also be engaged in tumors to promote their growth.

YPSA-5

Mice with Syngeneic Human Immune System and Liver to Study Cellular Immunity Against Viral Hepatitis

Eva Billerbeck[1,*], Michiel C. Mommersteeg[1,*], Amir Shlomai[1], Jing W. Xiao[1], Linda Andrus[1], Ankit Bhatta[1], Marcus Dorner[1], Anuradha Krishnan[2], Michael R. Charlton[2], Luis Chiriboga[3], Charles M. Rice[1], and *Ype P. de Jong*[1,4]
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Most pathogens that cause human disease cannot infect rodents. Engrafting human tissues in mice is one strategy to overcome this species barrier. For example, human liver chimeric mice are useful models to study hepatitis B (HBV), hepatitis C virus (HCV) and malaria infections. Independently, immunodeficient mice reconstituted with CD34+ hematopoietic stem cells (HSC) derived from fetal liver reliably develop human T and B lymphocytes and are permissive for HIV infection. Combining these systems using syngeneic grafts has long been hampered by inefficient liver reconstitution with human fetal hepatoblasts. Here we describe the efficient engraftment of immunodeficient fah-/- mice with syngeneic human fetal hepatoblasts and HSC. Addition of human oncostatin-M enhanced human hepatoblast engraftment by 5-100 fold. Mice highly engrafted with hepatoblasts could support chronic HCV and HBV infection. HBV viremia but not HBsAg production could be suppressed by entecavir treatment, similar to what is observed in patients. In contrast to mice singly engrafted with HSC, which predominantly develop human T and B lymphocytes, mice co-transplanted with syngeneic hepatoblasts also contained physiological levels of intrahepatic human monocytes and natural killer (NK) cells. Upon infection with HBV, these mice displayed rapid and sustained viremia and an expansion of NK cells in their blood and liver, mimicking observations of acute HBV infection in man. In conclusion we have generated a new mouse model with syngeneic human immune system and liver that displays an improved human immune cell repertoire and can be used to study viral hepatitis.

YPSA Poster Abstracts

YPSA-6

Identification of Aiolos as a Novel Regulator of Eosinophil Development

Carine Bouffi[1], Andrey V. Kartashov[1], Kaila L. Schollaert[1], Artem Barski[1,2], and *Patricia C. Fulkerson*[1]

[1]Division of Allergy and Immunology, [2]Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Department of Pediatrics, University of Cincinnati, Cincinnati, OH, USA

The production of mature eosinophils is a tightly orchestrated process to sustain normal eosinophil levels in tissues, while maintaining low numbers of these complex and sensitive cells in the blood; yet, the regulation of homeostatic eosinophil production is ill-defined. We took a global approach to identify genome-wide transcriptome and epigenome changes that occur during homeostasis at critical developmental stages, including eosinophil-lineage commitment (eosinophil progenitor [EoP] compared to granulocyte-monocyte progenitor [GMP]) and eosinophil maturation (eosinophil compared to EoP). Our analyses revealed markedly greater transcriptome alterations associated with eosinophil maturation (1,199 genes) compared to eosinophil-lineage commitment (490 genes). Our analyses also delineated a 976 gene eosinophil-lineage transcriptome that included a repertoire of 56 transcription factors, many of which have never previously been associated with eosinophils. EoPs and eosinophils, but not GMPs, expressed Helios and Aiolos, members of the Ikaros family of transcription factors. Aiolos-deficiency resulted in increased eosinophil frequency in the bone marrow, highlighting a role for Aiolos in eosinophil development and/or eosinophil mobilization. Further, Aiolos and Helios binding sites were significantly enriched in genes with active chromatin marks and expressed by EoPs and eosinophils, suggesting roles for these TFs in directing eosinophil development. We also noted a significant enrichment for Aiolos binding motifs in genes that encode for TFs, including Stat3 , Nfatc1 , Nfkb1 , Irf1 , and Irf2 , in the eosinophil-lineage transcriptome, suggesting that Aiolos binding may be an important hierarchical step in the progression of eosinophil maturation. Together, our study reveals that the dynamic changes in gene expression associated with eosinophil development include novel transcriptional regulators, such as Aiolos and Helios. Comprehensive epigenomic and transcriptomic profiling during critical stages in eosinophil development will ultimately define the programming necessary for eosinophil development.

YPSA-7

Crosstalk Between AMPK in Myocytes and Fibroblasts Regulates Cardiac Fibrosis

***J. Travis Hinson*[1], Anant Chopra[2], Christopher S. Chen[2], Jonathan G. Seidman[3], and Christine E. Seidman[1,3,4]**

[1]Brigham and Women's Hospital; [2]Boston University; [3]Harvard Medical School; [4]Howard Hughes Medical Institute

Cardiac fibrosis is a dynamic process influenced by chemical and mechanical signals in healthy and diseased hearts. It is exquisitely regulated by complex cellular interactions, and yet our understanding of these factors remains incomplete. Here, we demonstrate a novel crosstalk between metabolic sensing in cardiomyocytes by AMP-activated protein kinase (AMPK) and fibroblast mediated fibrosis. In hearts and iPS-derived myocytes with activating mutations in PRKAG2 that cause left ventricular hypertrophy, fibrosis is inhibited unlike other forms of hypertrophic cardiomyopathy due to sarcomere mutations. Myocytes with activated AMPK transcribe less IGF-2 and CTGF, which are both necessary for fibroblast proliferation, canonical TGF-beta signaling and extracellular matrix production in vitro. In vivo, chemical activation of AMPK by A-769662 in a mouse model of hypertrophic cardiomyopathy due to MYH7 mutations also inhibits cardiac fibrosis. Our findings implicate AMPK as not only an intracellular metabolic hub in myocytes, but also a master communicator of metabolic stress to neighboring cells via inhibition of IGF-2 and CTGF production.

YPSA-8

Hopx Coordinates Bmp and Wnt Signaling to Regulate Specification of the Cardiomyoblast Lineage

***Rajan Jain*[1], Deqiang Li[1], Mudit Gupta[1], Kyoung-Jae Won[2], and Jonathan A. Epstein[1]**

[1]Department of Cell and Developmental Biology, Penn Cardiovascular Institute, Institute of Regenerative Medicine, and [2]Department of Genetics, Institute for Diabetes, Obesity, and Metabolism, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA

Cardiac progenitor cells are multipotent and give rise to cardiac endothelium, smooth muscle and cardiomyocytes. Here, we identify and characterize the cardiomyoblast progenitor that is committed to the cardiomyocyte fate and we characterize the niche signals that regulate commitment. Cardiomyoblasts express Hopx, which functions to coordinate local Bmp signals to inhibit the Wnt pathway, thereby promoting cardiomyogenesis. Hopx integrates Bmp and Wnt signaling by physically interacting with activated Smads and repressing Wnt genes. The identification of a committed cardiomyoblast that retains proliferative potential and characterization of signals required for commitment to the myocyte fate will inform future regenerative therapies for the heart. In addition, Bmp signals characterize adult stem cell niches in other tissues, including the intestine and hair follicle, where Hopx-mediated inhibition of Wnt is likely to contribute to stem cell quiescence and to explain the role of Hopx as a tumor suppressor.

YPSA Poster Abstracts

YPSA-9

Loss of mDia1 Mediates the Development of del(5q) MDS Through Upregulation of the Innate Immune Response and Induction of Neutropenia

Yang Mei, Baobing Zhao, Jing Yang, and *Peng Ji*
Department of Pathology, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

mDia1, the diaphanous homolog 1 of Drosophila in mouse, is a formin protein involving in the linear actin polymerization. Recently, our group reported that patients with del(5q) myelodysplastic syndromes (MDS) showed a significantly decreased mDia1 expression. Mice with mDia1 deficiency developed age related hematologic features mimicking human MDS. In these mice, CD14 is aberrantly overexpressed on granulocytes, which sensitized the innate immune response upon lipopolysaccharide (LPS) injection. Importantly, chronic LPS injection accelerated the development of MDS. Here we report that 1) the mDia1 KO mice also show a hypersensitive innate immune response to damage-associated molecular pattern molecules (DAMPs), which can be partially blocked by CD14/Toll-like receptor 4(TLR4) signaling pathway inhibitors. This is significant since release of DAMPs from necrotic or senescent cells is a common age-related phenomenon. DAMPs are also detected in cancers including MDS. Thus our study may shed lights on how the deregulated innate immune responses get involved in the pathogenesis of MDS in a more pathophysiologically relevant etiology. 2) mDia1 KO mice have increased Gr1/Mac1 expression on the granulocytes in peripheral blood. Mac-1, as the predominant β2 integrin on granulocytes, plays key roles in the adhesion of leukocytes to the endothelium, and regulates cell adhesion, migration, and chemotaxis. In this respect, the mDia1 knockout mice develop severe neutropenia, which is due to the upregulation of Gr1/Mac1 and increased binding of the cells to intercellular adhesion molecule-1 (ICAM-1). Finally, we revealed the mechanism of Gr1/Mac1 upregulation by showing that loss of mDia1 significantly affected the endocytosis of Gr1 and Mac1 on granulocytes. However, the mRNA levels of Gr1 and Mac1 were not affected. Taken together, these studies reveal the significance of loss of mDia1 in the pathogenesis of del(5q) MDS through upregulation of innate immune response and accelerated granulocyte clearance.

YPSA-10

Keratinocyte-associated IL-6 is Elevated in Cutaneous Lupus Rashes and Correlates with Renal Function in Systemic Lupus Erythematosus Patients

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Background: Systemic lupus erythematosus (SLE) is a devastating autoimmune disease characterized by diverse complications such as immune-complex mediated nephritis and scarring skin lesions. While some pathways which contribute to cutaneous disease, such as increased type I interferon (IFN) production, are known, the pathogenic mechanism driving lupus rashes remains unclear. Interleukin-6 (IL-6) is a pro-inflammatory cytokine which has gotten recent attention in SLE as IL-6 is increased in the serum of active patients and blockade of IL-6 is therapeutic in several murine lupus models. Further, several phase I human trials have suggested IL-6 blockade may be promising for treatment of SLE. However, the role which IL-6 plays in cutaneous lupus erythematosus (CLE) rashes remains unclear. **Methods:** All studies were approved by the University of Michigan Internal Review Board (IRB #72843). Formalin fixed, paraffin-embedded biopsies of CLE rashes were obtained from the University of Michigan Pathology database. RNA was isolated using an Optimum RNA extraction kit, reverse transcribed into cDNA and semiquantitative real-time PCR was used to determine the expression level of the myxovirus (influenza virus) resistance 1 (MX-1) and interleukin-6 (IL6) genes when normalized to expression of GAPDH. IL-6 staining of CLE biopsies was completed using immunohistochemistry. Chart review was used to determine serum creatinine (Cr) at the time of skin biopsy and at 2-3 years post cutaneous lupus diagnosis. **Results:** Real-time PCR analysis of subacute cutaneous lupus erythematosus (sCLE) (n=21) and discoid (DLE) (n=22) rashes demonstrated a significant upregulation of both the IFN-regulated gene, MX1, and the pro-inflammatory cytokine IL-6 when compared with control samples (n=9). There was a strong correlation between the upregulation of the interferon signature in the skin and the fold change in IL-6 levels within the biopsies ($p=0.004$). Importantly, however, only the fold change of IL-6 within the skin biopsy correlated with the presenting Cr or the follow up Cr at years 2-3. Immunohistochemical analysis of skin biopsies confirmed the upregulation of IL-6 when compared to control. The primary site of IL-6 upregulation was in the epidermis, and no differences were seen between subtypes of lupus biopsies. **Conclusions:** IL-6 is increased at the RNA and protein level within cutaneous lupus biopsies when compared to healthy control skin. The degree of IL-6 upregulation correlated with renal dysfunction, suggesting that perhaps the degree of cutaneous IL-6 dysregulation may predict renal involvement. Further, our data suggest that IL-6 upregulation occurs primarily within the keratinocyte population, suggesting that interactions with the environment may drive IL-6 dysregulation in CLE. Further investigations should focus on the pathogenic significance of IL-6 upregulation in the skin and whether targeting this pathway will have an impact on cutaneous disease activity.

YPSA Poster Abstracts

YPSA-11

Human Tumorigenesis Induced by Endogenous DNA Transposase

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Recent cancer genome surveys have revealed extremely low rates of coding gene mutations in distinct tumor subtypes, suggesting that alternative mechanisms must contribute to their pathogenesis. Transposons are mobile genetic elements that are found in all living organisms, including humans where they occupy nearly half of the genome. Their mobilization can cause structural rearrangements in normal and cancer cells. However, it remains unknown whether transposition is a cause of cellular transformation or merely a bystander effect of dysregulated gene expression. Here, we report that *PGBD5*, a recently characterized human gene related to the *piggyBac* transposase from the cabbage looper moth, is aberrantly expressed in rhabdoid tumors, medulloblastoma, acute leukemias, and some sarcomas and carcinomas. Ectopic expression of *PGBD5* in non-transformed primary human cells is sufficient to induce anchorage independence *in vitro* and penetrant tumor formation in immunodeficient mice *in vivo*. *PGBD5* expression is sufficient to induce genomic mobilization of engineered DNA transposons in human cells, and purified recombinant PGBD5 exhibits transposase domain-dependent endonuclease activity *in vitro*. Flanking-sequence exponential anchored PCR and massively parallel sequencing of DNA transposon integrations revealed distinct activity on piggyBac-like inverted terminal repeats, and preference for specific euchromatic human genomic loci. This enables mapping of structural rearrangements of endogenous human transposable elements in primary human tumor genomes, some of which target genes involved in cellular transformation. We find that PGBD5 transposase-induced cell transformation is associated with morphologic de-differentiation, induction of distinct Polycomb gene expression programs and structural chromatin remodeling, consistent with its epigenetic control. These findings reveal an unanticipated mechanism of human tumorigenesis, genomic plasticity and structural alterations of non-coding regulatory genomic loci in human cancer.

YPSA-12

Genomic Evaluation of AML Clearance After Induction Chemotherapy to Assess Risk of Relapse

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Achievement of complete remission following induction chemotherapy is the initial goal for patients with acute myeloid leukemia (AML). Unfortunately, up to 20% of patients fail to respond to induction chemotherapy, and approximately 50% of all patients experience relapse, both of which lead to lower overall survival. While some factors are known to predict outcome in AML (e.g. conventional karyotyping, age, and white blood cell count), the use of deep digital sequencing has not been fully investigated as a potential approach for the prediction of relapsed disease. We characterized the exome (n=25) or genome (n=25) of 50 adult patients with de novo AML (6 favorable risk, 32 intermediate risk, 12 unfavorable risk) that entered complete remission at ~day 30 following induction chemotherapy, and then asked whether there was an association between the detection of variants after chemotherapy and event-free survival (EFS). We were able to follow a mean of 12.0 variants (range 5-30) per patient by targeted deep sequencing. At least one leukemia-associated variant was detected in at least 5% of cells in the remission sample in 24 out of 50 cases at ~day 30. This was associated with an inferior EFS compared to the 26 patients with no detectable variants; those with detectable variants had a median EFS of 6.0 months versus an EFS of 17.9 months (p=0.0001, Log-rank test) in patients without detectable variants. Similar findings were observed when considering only the 32 intermediate risk patients (median EFS of 8.8 versus 25.6 months, p=0.003). Multivariate analysis revealed that this test was associated with a reduced EFS (hazard ratio 3.32, p=0.005) and overall survival (hazard ratio 2.88, p=0.03) in intermediate risk cases. Activating mutations in FLT3, NRAS and/or KRAS were nearly universally cleared, while variants in DNMT3A, TET2 and IDH1/IDH2 were more likely to persist in the post induction samples. These data provide a framework for using genomic data to predict risk of relapse in patients with AML.

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Antiphospholipid Antibodies Promote the Release of Neutrophil Extracellular Traps: A New Mechanism of Thrombosis in the Antiphospholipid Syndrome

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Objective: Antiphospholipid antibodies (aPL), especially those targeting beta-2-glycoprotein I (β2GPI), are well known to activate endothelial cells, monocytes and platelets, with prothrombotic implications. In contrast, the interaction of aPL with neutrophils has not been extensively studied. Neutrophil extracellular trap (NET) release has recently been recognized as an important activator of the coagulation cascade, as well as an integral component of arterial and venous thrombi. Here, we hypothesized that aPL activate neutrophils to release NETs, thereby predisposing to the arterial and venous thrombosis inherent to the antiphospholipid syndrome (APS). **Methods:** Neutrophils, sera, and plasma were prepared from patients meeting criteria for primary APS by the revised Sapporo classification criteria (n=52), or from healthy volunteers. No patient in this study carried a concomitant diagnosis of systemic lupus erythematosus. **Results:** Sera and plasma from patients with primary APS have elevated levels of both cell-free DNA and NETs, as compared to healthy volunteers. Freshly-isolated neutrophils from patients with primary APS are predisposed to high levels of spontaneous NET release, while APS-patient sera, as well as IgG purified from APS patients, stimulate NET release from control neutrophils. Human aPL monoclonal antibodies, especially those that target β2GPI, also enhance NET release. The induction of NETs is abrogated with inhibitors of reactive oxygen species formation and toll-like receptor 4 signaling. Highlighting the potential clinical significance of these findings, aPL-mediated NETs promote thrombin generation. **Conclusion:** Neutrophil NET release warrants further investigation as a novel therapeutic target in APS.

YPSA-14

X Chromosome Genes and Male Cancer Predominance

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Many cancer types are more prevalent among males, but this sex bias is not completely understood. We hypothesized that X chromosome mutations may be associated with cancer sex-predominance. We first sequenced DNA from blastic plasmacytoid dendritic cell neoplasm (BPDCN), an aggressive leukemia with significant male predominance (>3:1 M:F). The splicing factor *ZRSR2*, located on chr.X, had acquired loss-of-function mutations in ~35% of BPDCNs, strikingly all from males. This observation highlighted a conundrum. Although females have two X chromosomes, one copy is inactivated in all female cells during embryogenesis. Therefore, cancer-associated mutations in chr.X tumor suppressors should be equally likely in males and females, because both have only one active copy of each gene. However, a small number of genes escape X-inactivation in female cells and are expressed from both maternal and paternal alleles. *ZRSR2* is one such escape gene. We postulated that tumor suppressor genes escaping from X-inactivation may protect females from certain cancers, and this may contribute to male cancer predominance. We propose to call these Escape from X Inactivation Tumor Suppressor (EXITS) genes. To query for EXITS genes in an unbiased manner, we designed computational algorithms to test for male predominance of chr.X loss-of-function mutations in >5500 cancers of 26 types from The Cancer Genome Atlas (TCGA). We discovered putative EXITS genes that were mutated more often in males than females, including *ATRX, DDX3X, MAGEC3, KDM5C,* and *KDM6A*. Male-predominant loss-of-function mutations were enriched for genes that escape X-inactivation. Mutations in EXITS genes were also significantly associated with male-predominant cancers, including glioma, melanoma, kidney, and bladder carcinoma. These data suggest that genes escaping X-inactivation provide protection from cancer development in females, and that a portion of the male predominance of certain cancers may be due to the overrepresentation of EXITS gene mutations in men.

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YPSA-15

Genetic Risk Prediction of Atrial Fibrillation and Implications for Identifying Ischemic Stroke Mechanisms

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Background: Atrial fibrillation (AF) is heritable and associated with an increased risk of stroke. We assessed whether AF genetic risk associates with A) incident AF beyond clinical risk factors, and B) ischemic stroke in patients without known AF. **Methods:** We derived genetic risk scores for AF by identifying non-redundant AF-associated single nucleotide polymorphisms (SNPs) from genome-wide association studies of individuals of European ancestry (6,707 with AF and 52,426 without). We summed AF risk allele dosages weighted by the log-relative risk to create scores. We tested different a priori scores for association with incident AF in 5 independent prospective studies (1,852 AF events, 16,164 total) after adjustment for clinical AF risk factors. We then tested scores for association with ischemic stroke in an independent case-control sample (839 cases, 3164 controls). **Results:** Genetic risk scores were based on various a priori combinations of 719 identified non-redundant SNPs. AF genetic risk associated with incident AF beyond clinical risk factors (P-value range 1.5x10-15–3.9x10-33 for different a priori SNP combinations). AF genetic risk also associated with ischemic stroke. As expected, scores were most significantly associated with the cardioembolic stroke subtype (n=289). Associations persisted after excluding individuals with known AF. For example, individuals without known AF and with the highest quartile of AF genetic risk had an increased odds of cardioembolic stroke (OR 1.77, 95% CI 1.07-2.91, P=0.001) as compared to those in the lowest quartile, using a score comprised of 123 SNPs each associated with AF in the original genome-wide association study at P≤1x10-4. **Conclusions:** AF genetic risk associates with incident AF beyond traditional clinical AF risk factors and associates with cardioembolic stroke even in those without known AF. These findings suggest that AF genetic risk may facilitate the identification of subclinical AF. Future studies will be directed at targeting high-risk stroke survivors for intensive monitoring for AF and potentially intensifying stroke prevention therapy.

YPSA-16

Lymphocyte Adaptor Protein, LNK, Deficiency Exacerbates Hypertension and End-organ Inflammation

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The lymphocyte adaptor protein LNK (also known as SH2B3) is primarily expressed in hematopoietic and endothelial cells where it functions as a negative regulator of cytokine signaling and cell proliferation. Single nucleotide polymorphisms in the gene encoding LNK are associated with autoimmune and cardiovascular disorders; however, it is not known how LNK contributes to hypertension. Here, we determined that loss of LNK exacerbates angiotensin II (Ang II)-induced hypertension and the associated renal and vascular dysfunction. At baseline, kidneys from Lnk-/- mice exhibited greater levels of inflammation, oxidative stress, and glomerular injury compared to wild type animals, and these parameters were further exacerbated by Ang II infusion. Aortas from Lnk-/- mice exhibited enhanced inflammation, reduced nitric oxide levels, and impaired endothelial-dependent relaxation. Bone marrow transplantation studies demonstrated that loss of LNK in hematopoietic cells is primarily responsible for the observed renal and vascular inflammation and predisposition to hypertension. Ang II infusion increased interferon gamma (IFNγ)-producing CD8+ T cells in the spleen and kidneys of Lnk-/- mice compared to WT mice. Moreover, IFNγ deficiency resulted in blunted hypertension in response to Ang II infusion. Together, these results suggest that LNK is a potential therapeutic target for hypertension and its associated renal and vascular sequela.

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YPSA-17

Krüppel-like Factor 6 Regulates Mitochondrial Function in the Kidney

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The primary etiology of chronic kidney disease is a direct consequence of initial dysfunction of the renal filtration barrier. Podocytes are terminally differentiated epithelial cells whose major function is the maintenance of this barrier. Podocyte injury is implicated in many kidney diseases including Focal Segmental Glomerular Sclerosis (FSGS) and HIV-associated nephropathy (HIVAN). Mitochondrial injury is observed with podocyte dysfunction, but mechanism(s) mediating the regulation of the mitochondrial injury upon podocyte stress are unclear. Krüppel-like factors (KLFs), zinc-finger DNA-binding transcription factors, play a critical role in epithelial cell biology. To ascertain the role of KLFs in podocyte injury, we utilized the murine model of HIVAN, where the expression of HIV-1 transgene results in significant podocyte injury. The expression of KLF transcripts was screened in primary podocytes isolated from wild-type mice and HIV-1 transgenic mice, revealing a significant reduction in KLF6 expression in HIV-1 transgenic mice compared to wild-type mice. We also observed that a podocyte-specific deletion of Klf6 in mice increased the susceptibility to FSGS. These changes were mediated by mitochondrial injury, demonstrated with increased mitochondrial fragmentation and reduced mitochondrial membrane potential, ATP levels, and oxygen consumption. Furthermore, putative binding sites for KLF6 were present in the promoter region of SCO2, cytochrome c oxidase (COX) assembly gene, by promoter analysis and chromatin immunoprecipitation. Our studies also demonstrated that KLF6 is an early inducible injury response gene that is critical to the maintenance of the COX assembly complex, thereby abrogating the release of cytochrome c and activation of apoptosis upon cell stress. Finally, we observed a podocyte-specific reduction in KLF6 expression in biopsy specimens from patients with FSGS as compared to healthy donor nephrectomies. These findings suggest that KLF6 is an early inducible injury response gene, critical to the maintenance of mitochondrial function and preventing apoptosis upon stress to the podocyte.

YPSA-18

Intracellular Control of the Human Fungal Pathogen, *Candida glabrata*, is Dependent on Spleen Tyrosine Kinase

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Invasive infection due to *Candida glabrata* carries a mortality of up to 40%. Elimination of intracellular fungal pathogens is a critical process performed by tissue macrophages. Following recognition by surface pattern-recognition receptors, fungal pathogens are phagocytosed into newly formed organelles termed phagosomes, which, in turn, are "matured" through the recruitment of host proteins resulting in acidification and activation of proteolytic enzymes. Spleen tyrosine kinase (syk) is essential for maturation of phagosomes containing the fungal carbohydrate, beta-1,3-glucan. The absence of syk activity interrupted phagolysosomal fusion resulting in arrest at a neutral, early stage phagosome. In the current study, we sought to define the contribution of syk to the control of phagocytosed live *Candida glabrata* in primary macrophages. In order to accurately measure intracellular yeast division, we designed a carboxyfluorescein succinimidyl ester (CFSE) yeast dilution assay. To assess potential effects of the fluorescence labeling process on yeast, growth kinetics of CFSE-labeled *C. glabrata* was compared to control at 30C. Using turbidity (OD600), there was no kinetic growth difference between CFSE-labeled versus unlabeled yeast cells. Following phagocytosis by macrophages, CFSE-labeled *C. glabrata* were recovered using detergent lysis, labeled using ConA-AF647 and subjected to two-color flow cytometry to assess division by CFSE dilution. *C. glabrata* remain undivided for up to 12 hours in co-culture with primary macrophages. In contrast, treatment of macrophages with inhibitors of src (PP2) or syk (R406) kinases resulted in loss of intracellular control of *C. glabrata* with initiation of division within 4 hours. R406 applied for short periods followed by washout suggests that syk activity within the first 10 min following phagocytosis is critical for control of *C. glabrata* division. In conclusion, using a novel CFSE dilution method to assess division, our results suggest that early syk activity following phagocytosis is essential for macrophage control of *C. glabrata*.

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Elevated Pulmonary Arterial Aldosterone Levels in Pulmonary Arterial Hypertension Activate Raptor to Promote Pulmonary Smooth Muscle Cell Proliferation and Apoptosis Resistance

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In pulmonary arterial hypertension (PAH), elevated levels of aldosterone (ALDO) induce vascular hypertrophic remodeling that is characterized by abnormal pulmonary vascular smooth muscle cell (PSMC) proliferation. Upregulation of the mammalian target of rapamycin complex 1 subunit Raptor promotes PSMC growth and survival; however, the factors regulating Raptor in PAH are not known. We hypothesized that hyperALDO in PAH activates Raptor to induce PSMC proliferation and apoptosis resistance. To test this hypothesis, PSMCs were exposed to vehicle (V) control or ALDO (10^{-7} mol/l) for 1 h. Compared to V-treated cells, ALDO increased expression of P-Raptor(Ser792) (19.4 ± 7.1 vs. 32.1 ± 2.2 units, $p<0.05$) and the Raptor target P-p70S6K(Thr389) (56.8 ± 3.1 vs. 84.6 ± 4.8 units, $P<0.01$) without affecting total Raptor/p70S6K levels. To confirm the role of Raptor in ALDO-p70S6K signaling, PSMCs were transfected with si-Raptor, to decrease Raptor expression by 66% ($P<0.04$), and exposed to ALDO; compared to untransfected cells, Raptor downregulation decreased P-p70S6K(Thr389) expression by 60% ($P<0.02$). Raptor activation by ALDO increased PSMC proliferation by 19% ($P<0.01$) and decreased apoptosis by 60% ($P=0.02$) as assessed by BrdU incorporation and apoptosis ELISA, respectively. To demonstrate the relevance of ALDO-Raptor signaling in PAH, PSMCs were treated with human pulmonary arterial plasma from PAH patients with confirmed hyperALDO or controls (232 ± 17 vs. 120 ± 42 ng/dl, $P<0.01$). PSMCs treated with 10% PAH plasma demonstrated a 1.7-fold ($P<0.01$) increase in the P-p70S6K(Thr389)/p70SK6 ratio compared to V-treated cells. In turn, ALDO inhibition with either spironolactone (10 μM) or eplerenone (10 μM) decreased P-p70S6K(Thr389) levels in ALDO- and PAH plasma-treated PSMCs by 38% ($P<0.05$) and 39% ($P<0.01$), respectively, to decrease cell proliferation by 56% ($P<0.03$). These data demonstrate that pulmonary arterial ALDO levels in PAH are sufficient to activate Raptor-p70S6K signaling to promote PSMC proliferation and apoptosis resistance *in vitro*. Regulation of Raptor by ALDO represents a novel mechanism to account for pulmonary vascular remodeling, and has therapeutic implications for patients with PAH and other diseases of similar pathobiology.

YPSA-20

Rational Development and Characterization of Humanized Anti-EGFR Variant III Chimeric Antigen Receptor T Cells for a Phase I Trial in Glioblastoma

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Chimeric antigen receptors (CARs) are synthetic molecules designed to re-direct T cells to specific antigens. CAR-modified T cells can mediate long-term durable remissions in B cell malignancies, but expanding this platform to solid tumors requires the discovery of novel surface targets with limited expression in normal tissues. The variant III mutation of the epidermal growth factor receptor (EGFRvIII) results from an in-frame deletion of a portion of the extracellular domain, creating a neoepitope. In glioblastoma, the EGFRvIII mutation is oncogenic, portends a poor prognosis, and is thought to be enriched in glioblastoma stem cells. However, because the neoepitope of EGFRv III is based on a small peptide sequence, an antibody or single-chain variable fragment (scFv) directed to this epitope must be rigorously tested to confirm lack of cross-reactivity to the ubiquitously expressed wild-type EGFR. We chose a vector backbone encoding a second generation CAR based on efficacy of a murine scFv-based CAR in a xenograft model of glioblastoma. Next, we generated a panel of humanized scFv's and tested their specificity and function as soluble proteins and in the form of CAR-transduced T cells; a low affinity scFv was selected based on its specificity for EGFRvIII over wild type EGFR. The lead candidate scFv was tested *in vitro* for its ability to direct CAR-transduced T cells to specifically lyse, proliferate, and secrete cytokines in response to antigen-bearing targets. We further evaluated the specificity of the lead CAR candidate *in vitro* against EGFR-expressing keratinocytes and *in vivo* in a novel model of mice grafted with normal human skin; a cetuximab-based CAR served as a positive control, and demonstrated activity against grafted human skin. EGFRvIII-directed CAR-T cells were also able to control tumor growth in xenogeneic subcutaneous and orthotopic models of human EGFRvIII+ glioblastoma. Based on these results, we have designed a phase I clinical study of CAR T cells transduced with humanized scFv directed to EGFRvIII in patients with either residual or recurrent glioblastoma (NCT02209376).

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YPSA-21

Frequent Inactivation of the Tumor Suppressor Gene FAT1 in Head and Neck Squamous Cell Carcinoma Promotes Tumor Growth and Engenders Novel Therapeutic Vulnerabilities

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Head and neck squamous cell carcinoma (HNSCC) is a lethal disease with survival rates that have stalled, despite an increasingly comprehensive catalog of the genetic alterations occurring in these tumors. We analyzed data from 21 cancer sites in The Cancer Genome Atlas and identified frequent loss-of-function alterations in the gene FAT1, most strikingly in HNSCC, in which 24% of tumors harbor mutation and 6% have homozygous deletion. Most mutations were predicted to be inactivating. We then investigated the function of FAT1 in a panel of HPV-positive and HPV-negative HNSCC cell lines which were assayed for mutational or copy number alterations in 280 HNSCC-relevant genes with massively parallel sequencing. Using transcriptomic and in vitro functional assays, we found that FAT1 knockdown strongly activates Wnt signaling and allied mitogenic pathways, resulting in growth, cell cycle progression, and an invasive phenotype. In FAT1-inactivated cells, overexpression of FAT1 reliably reversed this phenotype. We used co-IP and reporter assays to show that FAT1 binds to beta-catenin and regulates its translocation to the nucleus, thereby suppressing transcriptional activity. These findings define a novel subclass of HNSCC, comprising the 30% of tumors in which FAT1 is inactivated, and connect this genetic subclass to upregulated Wnt signaling, a heretofore unappreciated signaling pathway that drives HNSCC and represents a therapeutic target for the emerging class of agents targeting Wnt.

YPSA-22

Regulation of Alkylation Damage Resistance by a Novel Deubiquitinase Complex

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Repair of DNA alkylation damage is critical for genomic stability and involves multiple conserved enzymatic pathways. Alkylation damage resistance, which is commonly observed in numerous different tumors, depends on the overexpression of alkylation repair proteins. However, the mechanisms responsible for this upregulation are largely unknown. Here, we show that an OTU domain deubiquitinase, OTUD4, is master regulator of multiple alkylation repair pathways. OTUD4 serves as positive regulator of MGMT, ALKBH2 and ALKBH3, three repair enzymes critical for reversing DNA alkylation damage. Remarkably, we find that OTUD4 catalytic activity is completely dispensable for this function. Rather, OTUD4 is a scaffold to recruit USP7 and USP9X, two deubiquitinases that in turn act catalytically on the alkylation repair proteins. Moreover, we show that loss of OTUD4, USP7 or USP9X in various tumor cells results in significantly greater sensitivity to alkylation damage. Consistently, a recently identified small molecule inhibitor of USP9X acts synergistically with alkylating agents to kill tumor cells. Taken together, this work reveals a novel, noncanonical mechanism by which an OTU family deubiquitinase regulates its substrates, and provides multiple new targets for alkylation chemotherapy sensitization of tumors.

YPSA-23

Transcription Factor Kruppel-like Factor 2 Mediates the Antithrombotic Effect of Bortezomib and is a Critical Determinant of Vascular Thrombosis

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Cancer-associated thrombosis is a major source of morbidity and mortality. Current paradigms indicate that the interaction between vascular endothelium and blood elements (platelets, myeloid cells) is critical for thrombosis but the molecular determinants operative in these cell types remain poorly understood. While previous studies from our laboratory and others have identified Kruppel-like transcription factor 2 (KLF2) as a key regulator of endothelial and hematopoietic cell biology, its role in thrombosis is unknown. We find that global postnatal deletion of KLF2 (GL-K2-KO) is strongly prothrombotic in both arterial and venous mouse models. Conversely, global overexpression of KLF2 (GL-K2-TG) conferred an anti-thrombotic effect without altering hemostasis. Cell-specific (endothelial, myeloid, and platelet) deletion of KLF2 indicates a dominant role for the myeloid cells in the thrombotic process. Clinical observations suggest that the proteasome inhibitor bortezomib confers potent antithrombotic properties but the molecular basis remains poorly understood. Cell culture studies show that bortezomib induces KLF2 in hematopoietic and endothelial cells. Further, wild type mice treated with non-myelosuppressive doses of bortezomib have a prolonged time to thrombosis. However, this effect is abrogated in the KLF2-deleted state confirming that the thromboprotective effect noted with bortezomib is KLF2-dependent. Collectively these studies identify KLF2 as a nodal determinant of vascular thrombosis. Further, they illuminate the molecular basis of the antithrombotic effect noted with bortezomib. Finally, the identification of a factor that alters thrombosis without affecting hemostasis has the potential to direct future antithrombotic strategies.

YPSA Poster Abstracts

YPSA-24

Group 2 Innate Lymphocytes are Early Effectors that Cooperate with Adaptive Immunity After Helminth Infection

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Helminth parasites infect up to two billion people, overwhelmingly in resource-limited countries, and eradication efforts have had limited success. Infections are usually chronic and biased toward a "type 2" immune response, an inflammatory pattern that is also classically associated with allergic pathology. Modulating immunity to treat and prevent disease requires a more complete understanding of the cellular pathways involved. In inflamed tissues, T helper type 2 (Th2) cells produce cytokines that recruit and activate innate effector cells, which in turn alter the local environment to repel intruders and repair damage. We and others have described Group 2 innate lymphoid cells (ILC2s) as an important novel source of type 2 cytokines IL-5, IL-9, and IL-13 in mice and humans. We hypothesize that these cells are "first responders," uniquely poised from early development to detect tissue stress and shape ensuing T cell responses. However, without a method to specifically target ILC2s, it has been difficult to establish a non-redundant role for these cells. We generated an IL-5 reporter mouse with an embedded lineage tracker/deleter allele to study ILC2 function *in vivo*. In models of acute and chronic helminth infection, innate IL-5-reporting cells increase in number and in fluorescence intensity, corresponding with eosinophilia and Th2 cell induction. Analysis of cytokine expression in an IL-5/IL-13-dual reporter strain showed different expression patterns in different effector cell types. Deletion of the ILC2s and live imaging of infected lung revealed a unique role for these cells in recruitment of other effectors. Our data provide new insights into a highly cooperative innate-adaptive relationship at the initiation of type 2 immunity. Further characterization of these interactions is ongoing, with the goal of identifying novel targets in treating human disease.

YPSA-25

BECN1 Sorts Surface Amyloid Precursor Protein for Endolysosomal and Autophagic Degradation

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The metabolism of amyloid precursor protein (APP) to amyloid-beta (A-beta) is a critical event in Alzheimer disease (AD) pathogenesis and strategies to prevent amyloidogenic APP processing may provide novel therapeutic approaches for AD. Generation of A-beta and non-amyloidogenic APP metabolites occur in the endosomal/recycling pathway and plasma membrane, respectively, implicating the plasma membrane as a critical site of APP regulation. Recent studies suggesting that Beclin 1/BECN1, an essential autophagy and endosomal regulatory protein, is an important factor in A-beta secretion led us to hypothesize that BECN1 sorts plasma membrane APP for degradative trafficking in lieu of amyloidogenic metabolism. We identified novel interactions between plasma membrane APP and the autophagy/endosomal regulatory proteins BECN1, PIK3C3, UVRAG, ATG14L and ATG16L1. To study the effects of BECN1 specifically on the surface APP pool, we labeled surface APP in living cells and studied its downstream trafficking and metabolism. Using fluorescence microscopy, we found that BECN1 promotes internalization and localization of surface APP predominantly to endosomes and endolysosomes. However, a smaller fraction of surface APP trafficked to ATG16L1-positive autophagosomal precursors and LC3-positive autophagosome, implicating a role for BECN1-dependent plasma membrane autophagy in APP degradation. BECN1 overexpression reduced the secretion of surface APP metabolites and promoted surface APP degradation via lysosomes. Mapping studies using BECN1 and APP mutants indicated that the association between APP and BECN1 is primarily mediated by a small region of the evolutionary conserved domain (ECD) of BECN1 and is independent of the association of BECN1 and PIK3C3. Deletion of the APP association region of the BECN1 ECD prevented BECN1 from sorting surface APP for endolysosomal and autophagic degradation. In summary, our studies reveal a novel functional interaction between surface APP and BECN1 and its significance in the regulation of APP sorting from the cell surface for degradation.

YPSA-26

Loss of One TACI Allele Reveals Haploinsufficiency at Late-stages of B-cell Development

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Background: Heterozygous C104R or A181E TACI mutations impair removal of autoreactive B cells, weaken B-cell activation and convey to Common variable immune deficiency (CVID) patients an increased risk for autoimmunity. How mutant TACI influences the function of wildtype TACI is unclear; different experimental models have suggested either a dominant-negative effect or haploinsufficiency. Objective: We investigated potential TACI haploinsufficiency by analyzing antibody-deficient Smith-Magenis Syndrome (SMS) patients, who possess only one TACI allele and antibody-deficient patients carrying one c.204insA TACI null mutation. **Methods:** We tested the reactivity of antibodies isolated from single B cells from SMS patients and patients with a c.204insA TACI mutation and compared them with counterparts from CVID patients with heterozygous C104R or A181E TACI missense mutations. We also assessed if loss of a TACI allele induced haploinsufficiency in naïve and memory B cells or recapitulated abnormal immunological features typical of CVID patients with heterozygous TACI missense mutations. **Results:** We found loss of a TACI allele did not impact TACI expression, activation responses or establishment of central B-cell tolerance in naïve B cells. Additionally, SMS patients and patients with a c.204insA TACI mutation displayed normal Treg function and peripheral B-cell tolerance. The lack of two TACI alleles did result in decreased TACI expression on memory B cells, resulting in impaired activation in vitro and antibody deficiency in vivo. Thus, loss of one TACI allele does not recapitulate autoimmune features of CVID-associated C104R and A181E TACI mutations, which likely encode dominant-negative products.

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YPSA-27

Modeling HBV Infection in Stem Cell Derived Hepatocytes Reveals Role of Perturbations in NTCP Expression and Genotype in HBV Permissiveness

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Worldwide, hepatitis B virus (HBV) infection is the most common viral hepatitis having infected over two billion people and chronically infecting more than 400 million, putting them at increased risk to develop cirrhosis and hepatocellular carcinoma. HBV research has been hampered by the virus's narrow host range and tropism for hepatocytes, which have led to a paucity of robust infectious systems for HBV. The current model systems to study HBV are hepatoma cell lines which have undergone a variety of genetic and metabolic changes. Consequently, major components of the viral entry process and viral life cycle and many aspects of virus-host interactions have been poorly understood. We have recently shown that micropatterned co-cultures of primary human hepatocytes with stromal cells and inducible pluripotent stem cells differentiated into hepatocyte-like cells (iHeps) are permissive to HBV infection. iHeps become permissive only during late stages of differentiation, following the appearance of the HBV receptor, sodium-taurocholate co-transporter polypeptide (NTCP). Mutations of NTCP (ie. Arg252His) have been shown to severely impair bile acid uptake but the impact that particular variants play in HVB infection is largely unknown. A gene editing approach using CrispR (and guide RNA's targeting the NTCP locus) along with homology directed repair enabled the production of induced pluripotent stem cells (iPS) with variant mutations in NTCP in the genomic NTCP locus. Wild-type and variant NTCP iPS were differentiated into iHeps and then inoculated with HBV containing serum. NTCP variants had variable permissiveness for HBV infection. In particular the Ser267Phe mutation impacts NTCP expression and HBV permissiveness to HBV infection. These results exemplify the utility of physiologically relevant infectious systems for studying HBV interactions with host cell genetics and physiology. Combining these systems with gene editing technologies will enable the dissection of clinically relevant mutations in physiologically relevant human infectious systems.

YPSA-28

Angiopoietin-like 4 is a Potent Vascular Permeability and Angiogenic Factor and a Novel Therapeutic Target for Patients with Diabetic Eye Disease

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The anticipated rise in the global prevalence of diabetes will undoubtedly result in a concurrent increase in the number of patients with vision impairment from diabetic eye disease, already the most common causes of severe vision loss in the working-age population in the developed world. Clinical trials assessing the efficacy of therapies targeting vascular endothelial growth factor (VEGF) have demonstrated a major improvement in vision in a minority of diabetic patients. This underscores the need for a better understanding of the pathogenesis of both diabetic macular edema (DME) and diabetic retinopathy (DR). We observed that hypoxia is a driving force for DME and DR by stabilizing hypoxia-inducible factor (HIF)-1α in retinal Müller glial cells. Increased HIF-1α, in turn, promotes the transcription of cytokines (e.g., VEGF) secreted by Müller cells. Blocking hypoxic accumulation of HIF-1α with digoxin inhibits the promotion of retinal edema and neovascularization in animal models. Interestingly, Müller cells strictly require HIF—but not VEGF—to promote both vascular permeability and angiogenesis, implicating additional HIF-dependent factors in both DME and DR. Using gene expression analysis, we identify angiopoietin-like 4 (ANGPTL4) as a novel cytokine potently upregulated by HIF-1 in hypoxic Müller cells in vitro and in the ischemic inner retina in vivo. ANGPTL4 is critical and sufficient for the promotion of vessel permeability and angiogenesis in vitro and in vivo. Expression of ANGPTL4 was increased in the aqueous and vitreous of diabetic patients, independent of VEGF levels, correlated with the presence of diabetic eye disease, and localized to areas of DME and retinal neovascularization. Collectively, our results suggest that targeting both ANGPTL4 and VEGF may be necessary for effective treatment or prevention of diabetic eye disease and further provide the foundation for studies evaluating aqueous ANGPTL4 as a biomarker to help guide individualized therapy for diabetic eye disease.

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YPSA-29

Repurposing FK506 to Increase BMPR2 Signaling and Improve Pulmonary Arterial Hypertension — A Fast Track from Cells to People

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Rationale: In order to improve long-term survival in pulmonary arterial hypertension (PAH) new treatment approaches are necessary that target occlusive vasculopathy instead of vasoconstriction of pulmonary arteries. Identifying genes/pathways that unify different pathologies and etiologies in PAH is a crucial first step for drug development. The Bone Morphogenetic Protein Receptor 2 (BMPR2) pathway, originally described as the cause of familial PAH, has gained significant interest over the past years as a potential master switch in PAH and therefore presents a promising treatment target in PAH. Hypothesis: Increasing BMPR2 signaling using repurposed drugs might improve experimental and clinical PAH. **Methods:** We performed a High Throughput Screen of > 3600 FDA approved drugs to identify activators of BMPR2. The best activator, FK506 (Tacrolimus), was tested in patient cells as well as in three rodent models of PAH (BMPR2 deficient mice, Monocrotaline and VEGF-R2-hypoxia treated rats). Three end-stage PAH patients were subsequently treated with FK506 on a compassionate basis and a phase II safety and tolerability trial was initiated in stable PAH patients (ClinicalTrials.gov NCT01647945). **Results:** FK506 was found to restore normal BMPR2 signaling and function in patient pulmonary artery (PA) endothelial cells, inhibit proliferation and induce apoptosis in PA smooth muscle cells as well as prevent and reverse pulmonary hypertension in three experimental rodent models of PH. FK506 was well tolerated in three end-stage PAH patients and stabilized their clinical course. A phase II proof of concept, safety and tolerability trial is underway to evaluate the use of low dose FK506 in stable PAH and to identify patients who might respond best to FK506 depending on their impairment in BMPR2 signaling. **Conclusion:** With FK506 being a FDA approved drug, it was possible to relatively quickly, in approximately 2 years, translate the basic science discoveries from the bench to the clinic.

YPSA-30

Multi-isotope Imaging Mass Spectrometry Demonstrates the Limited Plasticity of Adult White Adipose Tissue in Mice and Humans

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Fat mass expansion occurs via two fundamental pathways, recruitment of new fat cells by differentiation of adipocyte progenitors or by hypertrophy of existing fat cells, the relative contributions of which may impact systemic metabolism and the development of diabetes mellitus. We recently established multi-isotope imaging mass spectrometry (MIMS) to address questions of cell fate and metabolism, with quantitative measurements of stable isotope tracers in sub-organelle domains. We have now applied MIMS to measure adipocyte turnover under normal homeostatic conditions and in an obesity model. In adult mice, we show depot-specific differences in homeostatic adipogenesis, with an order of magnitude higher rate in adult visceral white adipose tissue (vWAT) compared to the subcutaneous depot (sWAT). An obesigenic diet markedly increased adipogenesis in both the vWAT and sWAT compartments during post-natal development, whereas in adult mice, a high fat diet led to a modest hyperplastic response only in vWAT. Quantitative measures of sWAT adipocyte turnover were positively associated with parameters of improved metabolic function. The demonstration of a significant physiologic link between quantitative measures of adipogenesis and systemic metabolic function in mice provided additional rationale to perform similar measurements in humans. Here, we present early experience measuring adipogenesis in a first-in-human proof-of-principle MIMS study. We performed stable isotope pulse-chase studies in healthy adult volunteers, administering 15N-thymidine and/or 2H-water. Analyses of fat biopsy samples taken one month after 15N-thymidine infusion demonstrate 15N-labeled interstitial cells, but no 15N-labeled adipocytes (n=580 adipocyte nuclei, 6 subjects pooled). MIMS analysis of adipose specimens from a volunteer after a 30-day administration of 2H-water identified 2 highly labeled adipocytes of 188 analyzed, consistent with a 12.8% yearly rate of new adipocyte formation. Together, these data demonstrate the limited plasticity of white adipose tissue in mice and humans, while establishing MIMS as a powerful new tool for human biomedical research.

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YPSA-31

Exome-wide Association Study for Coronary Artery Disease

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Genome-wide association studies (GWAS) have identified 47 loci harboring DNA sequence variants robustly associated with risk of coronary artery disease (CAD). Almost all risk variants identified thus far are common and located in non-coding sequence, however, and as a result the causal variants and genes in most loci remain unclear. Low-frequency coding variants (i.e. those with minor allele frequency [MAF] between 0.1% and 5%) associated with risk of CAD have been successful in identifying causal genes and therapeutic targets. For example, the discovery of low-frequency coding variants in the proprotein convertase subtilisin/kexin type 9 (*PCSK9*) gene which protect against CAD through effects on low-density lipoprotein cholesterol has motivated the development of a new class of therapeutics. To date, no systematic search for such variants has been carried out. We designed a large-scale genotyping experiment to test the hypothesis that additional low-frequency coding variants are associated with risk of disease. We tested 54,003 nonsynonymous variants with minor allele frequency > 0.1% covering 13,715 human genes for association with CAD in up to 193,638 individuals. We confirmed known low-frequency associations in the *LPA* and *PCSK9* genes and discovered two nonsynonymous variants representing novel CAD loci. A missense variant in the sushi, von Willebrand factor type A, EGF and pentraxin domain containing 1 gene (*SVEP1*) was associated with increased CAD risk (p.D2702G with MAF=3.7%; odds ratio [OR] of disease for each copy of the rare allele=1.14; $p=4.2x10^{-10}$) while a low-frequency missense variant in the angiopoietin-like 4 gene (*ANGPTL4*) was associated with protection from CAD (p.E40K with MAF=2.1%; OR=0.86; $p=4.0x10^{-8}$). Subsequent sequencing of *ANGPTL4* in 9,731 individuals identified multiple rare null alleles collectively associated with a more pronounced protective effect from risk of CAD (OR of disease for carriers of any null allele=0.43; p=0.05). These results establish two additional low-frequency coding variants affecting CAD risk; overall, however, such variants seem to contribute minimally to the genetic architecture of CAD.

YPSA-32

Individualizing Risk Assessment for Type 2 Diabetes and Cardiovascular Diseases in Young Adults

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Risk assessment models for diabetes and cardiovascular disease have been mainly derived from studies of middle-aged populations. Defining the risk of adolescents and young adults to develop cardiometabolic diseases later in life remains a challenge. The MELANY cohort is an ongoing prospective follow-up study which provides an excellent opportunity to assess the progression to diabetes or coronary artery disease (CAD) among adolescents and young adults (aged 25-30 years). This population has been a subject of intense research to evaluate the role of 'classic' and novel risk factors for cardiometabolic outcomes. Following over 35,000 men from adolescence to adulthood we were able to analyze the impact of body-mass-index (BMI), fasting glucose and triglyceride levels and inflammatory markers on incidence rates of both diabetes and angiography proven CAD. During approximately 650,000 person-years of follow-up (mean follow-up, 17.4 years), 1173 incident cases of diabetes and 327 cases of CAD have been documented. In multivariate models adjusted for age, family history of diabetes/CAD, blood-pressure, lifestyle factors, and blood biomarkers, a significant increase in the risk of cardiometabolic diseases could be detected well within what is currently considered 'normal range' of both BMI, fasting glucose and triglyceride levels. Interaction models of the combined risk factors enabled the detection of sub-groups at an 8 to 10-fold increase in the risk for either diabetes or CAD that would have been otherwise missed by applying risk prediction models derived from older populations. In conclusion, elevated anthropometric (BMI), metabolic (glucose and triglyceride) and inflammatory (white blood cell count) parameters already well within the range currently considered normal constitutes a substantial risk factor for both type 2 diabetes and CAD in midlife. These results allow better detection of adolescents and young adults at increased cardiometabolic risk for whom early preventive interventions may be of benefit.

YPSA Poster Abstracts

YPSA-33

Downregulation of ERCC6: A Potential Mechanism for the Effects of PARP Inhibition in Malignant Peripheral Nerve Sheath Tumor

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Malignant peripheral nerve sheath tumors (MPNSTs) are devastating and intractable sarcomas. Surgical excision is the mainstay of MPNST therapy; however, MPNSTs often become unresectable, resulting in extremely poor patient outcomes. Consequently, this dismal prognosis calls for the development of effective therapies. MPNSTs are karyotypically-complex sarcomas; multiple mechanisms have been proposed to contribute to their genomic instability as well as the acquisition of molecular aberrations associated with neoplastic transformation. We propose that MPNST cells are defective in DNA repair and/or specific cell cycle checkpoints resulting in genetic instability, and hypothesize that this will render these cells susceptible to agents that induce additional DNA damage, such as PARP inhibitors. PARP inhibitors are hallmarked by particularly dramatic effects in BRCA-/- malignancies, as well as those with BRCAness. However, this genetic aberration (or any other that would indicate sensitivity to PARP inhibitors) has not been demonstrated in MPNST. Following this rationale, we evaluated PARP inhibition as a therapeutic strategy for MPNST and subjected a panel of MPNST cell lines to transcriptome profiling with RNA-Seq. Preclinical models indicated that MPNST cells were susceptible to PARP inhibition. We found that *ERCC6* (excision repair cross-complementing rodent repair deficiency, complementation group 6) encoding for CSB, a protein involved in transcription-coupled DNA repair, is significantly down-regulated in MPNST cells when compared to normal Schwann cells. Furthermore, lentiviral overexpression of CSB resulted in increased resistance to PARP inhibitors. Our data demonstrate that PARP inhibitors elicit marked anti-MPNST effects, and that this sensitivity is possibly due to a loss of CSB expression. The identification of this novel mechanism of PARP inhibitor sensitivity warrants further examination in prospective studies to determine the clinical and therapeutic utility in this clinical context.

YPSA-34

Molecular Evolution of *S. aureus* ST398

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Bacterial whole-genome sequencing has enabled an unprecedented resolution of the global spread of methicillin-resistant *Staphylococcus aureus* clones. However, little remains known about the clonal evolution of methicillin-susceptible *S. aureus* (MSSA). MSSA sequence type (ST)398 has emerged as a pandemic clone affecting both humans and livestock. Here, we used whole-genome sequencing of 287 ST398 isolates drawn from an epidemiological network of infection and colonization in northern Manhattan, the Caribbean and previously sequenced global collection of isolates to explore its short-term evolution and transmission patterns. Phylogenetic analysis predicted that human-associated ST398 MSSA diverged from a most common recent ancestor about 40 years ago and that it was likely introduced into New York City on multiple occasions. Using pair-wise single-nucleotide polymorphism (SNP) distances as a measure of genetic relatedness between isolates, we observed that ST398 subtypes clustered in both households and social networks, indicating their critical role as reservoirs for transmission and diversification. However, we observed a relatively high within-host SNP variability (mean SNP distance 12) compared to our previous studies on the dominant MRSA clone USA300 (mean SNP distance 2). We did not find evidence for hypermutability in ST398 or differences in clonal substitution rate between these two clones. Our results indicate that the dynamics of colonization, persistence and transmission differ substantially between USA300 MRSA and ST398 MSSA. By integrating whole-genome sequencing with detailed epidemiological analyses, our study provides an important framework for delineating the full diversity and spread of community MSSA and MRSA and other emerging pathogens in large urban community populations.

YPSA Poster Abstracts

YPSA-35

Maintenance of Active Chromatin by Long Noncoding RNAs (lncRNAs) — A Novel Mechanism for Regulation of Gene Expression

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The genome is extensively transcribed into long intergenic noncoding RNAs (lincRNAs), many of which are implicated in gene silencing. Potential roles of lincRNAs in gene activation are much less understood. Development and homeostasis require coordinate regulation of neighboring genes through a process termed locus control. Some locus control elements and enhancers transcribe lincRNAs, hinting at possible roles in long-range control. In vertebrates, 39 Hox genes, encoding homeodomain transcription factors critical for positional identity, are clustered in four chromosomal loci; the Hox genes are expressed in nested anterior-posterior and proximal-distal patterns colinear with their genomic position from 3′ to 5′ of the cluster. We have identified HOTTIP, a lincRNA transcribed from the 5′ tip of the HOXA locus that coordinates the activation of several 5′ HOXA genes in vivo. Chromosomal looping brings HOTTIP into close proximity to its target genes. HOTTIP RNA binds the adaptor protein WDR5 directly and targets WDR5/MLL complexes across HOXA, driving histone H3 lysine 4 trimethylation and gene transcription. Induced proximity is necessary and sufficient for HOTTIP RNA activation of its target genes. Thus, by serving as key intermediates that transmit information from higher order chromosomal looping into chromatin modifications, lincRNAs may organize chromatin domains to coordinate long-range gene activation.

YPSA-36

A Protein Kinase C Phosphorylation Motif in GLUT1 Affects Glucose Transport and is Mutated in GLUT1 Deficiency Syndrome

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Mutations in the facilitative glucose transporter GLUT1 cause a pediatric neurological disease characterized by epilepsy and movement disorders, GLUT1 deficiency syndrome. Several cases of this disease have mutations in a potential phosphomotif surrounding Serine 226 (S226). Through in-vitro kinase assays, mass spectrometry, and phosphospecific antibodies, we identify Serine 226 in GLUT1 as a PKC phosphorylation site. The phosphorylation of S226 is required for the rapid increase in glucose uptake and enhanced cell surface localization of GLUT1 induced by the phorbol ester 12-O-tetradecanoyl-phorbol-13-acetate (TPA). Endogenous GLUT1 is phosphorylated on S226 in primary endothelial cells in response to TPA and VEGF. The pathogenic R223P, R223Q, R223W, and 227-228insPPV mutations disrupt the PKC phosphomotif, impair the phosphorylation of S226 in vitro, and blunt TPA-mediated increases in glucose uptake. We demonstrate that phosphorylation of the GLUT1 transporter on S226 rapidly regulates glucose transport and propose that this modification is important in the physiological regulation of glucose transport.

YPSA Poster Abstracts

YPSA-37

The E3 Ligase Subunit FBXW12 Modulates the IL-22 Receptor Abundance and Host-Protective Epithelial Proliferation

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Signaling of the cytokine interleukin (IL)- 22 is necessary for the containment of pathogens in animal models of pneumonia and bacteremia from respiratory infections by *Klebsiella pneumoniae* and *Staph aureus*. The IL-22 receptor (IL-22R) is only expressed on epithelial cells and is prominent on mucosal surfaces, though cellular regulation of IL-22R is not well characterized. We have recently described IL-22 induced cortactin phosphorylation and processing of IL-22R through the ubiquitin proteasome system, which modulates IL-22 dependent responses. To identify specific cellular regulators of this receptor, we screened human and mouse IL-22R abundance in the presence of overexpressed F-box protein constituents of ubiquitin E3 ligases and identify that mIL-22R is shuttled for degradation by the previously uncharacterized E3 ligase subunit F-box protein W22 (FBXW22). Plasmid-derived FBXW22 decreases the cellular half-life of IL-22R and depletes both exogenous and native IL-22R in a dose-dependent fashion. We furthermore observe that mIL-22R is ubiquitinated *in vitro* in a cell free system with requisite ubiquitin conjugation machinery and FBXW22. The human IL-22R is destabilized by FBXW12, a human homolog of FBXW22. FBXW12 also depletes endogenous and plasmid-derived hIL-22R protein in human airway epithelia and associates with ubiquitin E3 ligase constituent Skp-1. FBXW12 shRNA knockdown causes increased IL-22R abundance and augments the second messenger STAT-3 phosphorylation in response to IL-22. Furthermore FBXW12 augments cell proliferation in the presence or absence of the IL-22 cytokine. These findings indicate that the heretofore undescribed proteins FBXW22 and FBXW12 function as E3 ligase constituents for ubiquitination and degradation of mouse and human IL-22R, respectively, and that FBXW12 functions as a growth suppressor in human cells. FBXW12 inhibition may therefore bolster host epithelial defense and infection containment to be an attractive therapeutic strategy for patients with pulmonary infection.

YPSA-38

Disease Progression on Ibrutinib Is Associated with the Acquisition and Evolution of Resistance Mutations

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The Bruton's Tyrosine Kinase (BTK) inhibitor ibrutinib is very effective in chronic lymphocytic leukemia (CLL) and has changed the standard of care for relapsed disease. We have previously shown that mutations in BTK at the binding site of ibrutinib and activating mutations in the downstream target of BTK, PLCγ2, can induce resistance to the drug in a cohort of 6 patients. We have evaluated 308 patients treated with ibrutinib at our institution on 4 consecutive clinical trials to determine whether these resistance mutations would be seen in a larger cohort. With a median follow-up of 20 months, 232 patients remain on therapy, 31 have discontinued because of progression, and 45 have discontinued for other reasons. Disease progression includes Richter's transformation or progressive CLL. Richter's appeared to occur early and CLL progressions later (cumulative incidence at 12 months: 4.5% (95% CI: 2.0% to 7.0%) and 0.3% (95% CI: 0% to 1.0%), respectively). Targeted deep sequencing with Ion Torrent technology on peripheral blood from 8 patients with Richter's transformation revealed 2 with mutations in BTK, and a lymph node sample showed no mutations in BTK or PLCγ2. Deep sequencing on 11 patients with CLL progression revealed BTK or PLCγ2 mutations in all. These mutations were not identified during pre-treatment in any patient. In patients where samples are available on therapy but pre-relapse, we can identify the emergence and subsequent amplification of these resistant clones, suggesting that these clones may be able to be targeted prior to overt disease progression. Median survival following Richter's transformation was 3.5 months (95% CI: 0.3-6.0), and 17.6 months (95% CI: 4.7-not reached) following CLL progression. CLL progression during ibrutinib therapy is therefore mediated by mutations in BTK and PLCγ2. Future clinical research will focus on therapeutic targeting of these resistant clones.

YPSA Poster Abstracts

YPSA-39

Twelve versus 30 Months of Dual Antiplatelet Therapy in Patients Undergoing Coronary Stenting for Acute Coronary Syndromes

***R.W. Yeh*[1,11], D.J. Kereiakes[2], S. Windecker[3], P.G. Steg[4], M. Rinaldi, A.H. Gershlick[5], D.E. Cutlip[6], D.J. Cohen[7], J.F. Tanguay[8], A. Jacobs[9], S. Wiviott[10], J.M. Massaro[11], A.C. Iancu[12], and L. Mauri[10,11] on behalf of the DAPT Study Investigators**

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Background: Patients presenting with acute coronary syndromes (ACS) have high risk of recurrent events. In the Dual Antiplatelet Therapy (DAPT) Study, continuing thienopyridine beyond 1 year reduced ischemic events after coronary stenting. We examined whether subjects presenting with and without ACS experienced similar event reductions with continued thienopyridine. **Methods:** The DAPT Study enrolled 25,682 subjects after coronary stenting. After 12 months of treatment with thienopyridine and aspirin, eligible subjects continued aspirin and were randomized to 18 months of continued thienopyridine or placebo. Randomization was stratified by baseline characteristics, including presentation with biomarker-positive ACS. The coprimary efficacy end points were stent thrombosis and major adverse cardiovascular and cerebrovascular events (MACCE; a composite of death, myocardial infarction, or stroke) during the period from 12 to 30 months, and the primary safety end point was moderate or severe bleeding. **Results:** Of 11,648 randomized patients, 3,576 (30.7%) presented with biomarker-positive ACS (47% ST-elevation myocardial infarction, 53% non-ST-elevation myocardial infarction). In patients with ACS, continued thienopyridine, as compared with placebo, reduced stent thrombosis (0.5% vs. 1.9%, HR 0.27, 95% CI 0.13-0.57, p<0.001) and m (3.9% vs. 6.8%, HR 0.56, 95% CI 0.42-0.76, p<0.001). Significant reductions in stent thrombosis and MACCE were also observed among non-ACS patients (stent thrombosis, 0.4% vs. 1.1%, HR 0.33, 95% CI 0.18-0.60, p<0.001; MACCE 4.4% vs. 5.3%, HR 0.83, 95% CI 0.68-1.02, p=0.08), although the reduction in MACCE was greater among ACS patients (interaction p=0.03). Continued thienopyridine increased bleeding in both ACS (1.9% vs. 0.8%, HR 2·38, 95% CI 1.27-4.42, p=0.005) and non-ACS patients (2.6% vs. 1.7%, HR 1.53, 95% CI 1.12-2.08, p=0.007) similarly (p = 0.21 for interaction). **Conclusions:** While patients with coronary stenting for ACS had greater risks of ischemic events than patients without ACS, the ischemic benefit of continued thienopyridine was robust for both ACS and non ACS subjects. Bleeding risks were similar.

YPSA-40

Loss of the SWI/SNF Component Arid1a Improves Mammalian Regeneration and Prevents Organ Damage

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The barriers to mammalian regeneration remain poorly understood. We show that chromatin-remodeling machinery regulates regenerative capacity and resistance to a broad range of injuries. Mice with deletion of Arid1a, a SWI/SNF component implicated in cancer, showed profoundly improved healing abilities after diverse surgical and chemical injuries. Liver-specific knockout (LKO) mice had increased hepatocyte proliferation, reduced tissue damage/fibrosis, and improved liver function after partial hepatectomy, toxic hepatocyte injury, and toxic bile duct injury. Within SWI/SNF complexes, Arid1a physically interacts with C/ebpα, a hepatocyte transcription factor that drives maturation and limits proliferation. Genome-wide analysis showed that loss of Arid1a reduces the recruitment and activity of C/ebpα on target promoters, resulting in expression programs that favor regeneration and cellular fitness during injury. Arid1a deficient mice also exhibited improved wound healing after ear hole punch and preserved haematopoietic function after cisplatin and irradiation, demonstrating that Arid1a is a regeneration suppressor in multiple tissues. Surprisingly, Arid1a LKO mice were also resistant to injury-induced liver carcinogenesis and conditional whole-body KO mice did not spontaneously develop cancer after one year. Our work shows that by inhibiting SWI/SNF, it is feasible to improve mammalian regenerative capacity without increasing cancer risk.

Howard Hughes Medical Institute Medical Fellows

The ASCI welcomes the following Howard Hughes Medical Institute Medical Fellows. They present their work at the ASCI Reception for New Members and Young Physician-Scientist Poster Session, on Saturday, April 25, 6 to 8 p.m.

Katie L. Anderson (HHMI-1)
College of Veterinary Medicine, University of Minnesota

Rima Shah Chakrabarti (HHMI-2)
Howard Hughes Medical Institute, University of Texas Southwestern Medical Center

Nan Guo (HHMI-3)
Stanford Institute of Stem Cell Biology and Regenerative Medicine

Jaewon J. Lee (HHMI-4)
Harvard Medical School and Tufts University School of Medicine

Sarah A. Low (HHMI-5)
Stanford University School of Medicine

E.C. Nakajima (HHMI-6)
University of Pittsburgh School of Medicine

Jong G. Park (HHMI-7)
Howard Hughes Medical Institute; Boston Children's Hospital, Harvard Medical School; and Duke University School of Medicine

HHMI-1

Combination Chemotherapy and CD47 Blockade Promote Melanoma Cell Phagocytosis, But are Not Sufficient to Create Anti-tumor Immunity

***Katie L. Anderson*[1], Debra Lins[2], Jens-Peter Volkmer[3], Irving L. Weissman[3], Matthew Mescher[2], Jaime Modiano[1,4]**
[1]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA; [2]Center for Immunology, University of Minnesota, Minneapolis, MN, USA; [3]Institute of Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Stanford, MN, USA; [4]Masonic Cancer Center, University of Minnesota, Minneapolis, MN, USA.

Melanoma cells demonstrate considerable resistance to immunotherapy using CD47 blockade as well as to doxorubicin chemotherapy. We hypothesized that a chemo-immunotherapy approach combining doxorubicin and CD47 blockade would mitigate this resistance. Specifically, we proposed that increasing "eat me" signals such as phosphatidylserine through chemotherapy would enhance the efficiency of tumor phagocytosis by macrophages and presentation of antigens to effector T cells. We confirmed that doxorubicin increased membrane phosphatidylserine exposure on 50-60% of B16 melanoma cells (B16-OVA) without altering membrane integrity. CD47 blockade caused a reproducible, albeit modest (2-3%) increase in phagocytosis of B16-OVA cells. Pretreatment with doxorubicin enhanced phagocytosis by an average of 10%; however, this increased phagocytosis did not consistently promote increased IL-2 or IFN-γ production by antigen-specific T cells. Neither activation of antigen-specific $CD8^+$ T cells in vivo nor a reduction in tumor burden was induced reproducibly by this chemo-immunotherapy approach. We conclude that expression of "eat me" signals by melanoma cells following chemotherapy renders them more susceptible to phagocytosis under conditions of CD47 blockade, but this effect does not promote an adaptive anti-tumor immune response. Additional work will be needed to define the mechanisms by which B16 melanoma cells resist chemo-immunotherapy.

HHMI Poster Abstracts

HHMI-2

A Bacterial Cholesterol Sensor to Assess Cholesterol Accessibility in Red Blood Cells

***Rima Shah Chakrabarti*[1,2], Arun Radhakrishnan[2], Jonathan C. Cohen[3], Helen H. Hobbs[1,2,3]**
[1]Howard Hughes Medical Institute, [2]Departments of Molecular Genetics and [3]Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX, USA

The only cells in the body that cannot synthesize cholesterol are red blood cells (RBCs), yet RBCs contain ~50% of circulating blood cholesterol. Whereas HDL is considered the major conduit for reverse cholesterol transport, we hypothesize that RBCs play a role in this pathway. To test this hypothesis, we developed an assay to measure accessible cholesterol in RBCs. We purified and fluorescently labeled domain 4 of a bacterial toxin, Anthrolysin-O (ALOD4), that binds membrane cholesterol. We incubated fALOD4 with RBCs from 164 healthy subjects and measured the fluorescence intensity using flow cytometry. The intra-assay and intra-individual variability were both <10%, whereas the inter-individual values varied over a 10-fold range. No correlation was found between fALOD4 binding and total RBC-cholesterol, hematocrit, or indices of RBC size. fALOD4 binding was inversely related to membrane phosphatidylcholine (PC) (-0.42, $p=6e^{-7}$) and directly related to lyso-phosphatidylcholine levels (LPC) (0.40, $p=6e^{-6}$). Phospholipase A2 treatment, which converts PC to LPC, increased binding 3-fold. fALOD4 binding did not correlate with plasma LDL-C levels, but was directly related to HDL-C (0.30, $p=6e^{-4}$), and inversely related to triglyceride levels (-0.57, $p=2e^{-12}$). Future studies will determine if variability in fALOD4 binding is intrinsic to RBC membranes, is genetically determined, or contributes to atherosclerosis.

HHMI-3

Bispecific Macrophage Enhancing Antibodies: A New Class of Cancer Immunotherapies that Target Myeloid Effector Cells

***Nan Guo,* Aaron M. Ring, Kipp Weiskopf, Jens-Peter Volkmer, Irving L. Weissman**
Stanford Institute of Stem Cell Biology and Regenerative Medicine

Myeloid effector functions are central to the immune response elicited by therapeutic antibodies and are emerging as attractive target pathways for cancer immunotherapy. CD47 is a myeloid-specific immune checkpoint upregulated by cancer cells to evade antibody directed cell-mediated cytotoxicity (ADCC) by neutrophils and antibody directed cell-mediated phagocytosis (ADCP) by macrophages. Agents that prevent CD47 from interacting with its myeloid receptor SIRPa robustly synergize with therapeutic monoclonal antibodies to enhance ADCC and ADCP of cancer cells. Thus, we hypothesized that bispecific agents that combined these activities into a single molecule could have emergent immunostimulatory properties. We termed these bispecific macrophage enhancing (BiME) antibodies, and we have created three agents to target either CD20, CD70, or HER2. Compared to their parent antibodies (rituximab, vorsetuzumab, and trastuzumab, respectively), the BiME antibodies elicited dramatically increased ADCC and ADCP across many cell lines, including Burkitt lymphoma, renal cell carcinoma, and breast adenocarcinoma.

HHMI-4

Inhibition of Epithelial Cell Migration and Breast Cancer Progression by SIRT3

***Jaewon J. Lee*[1,2,7], Mei Rosa Ng[1,a], Amey Barakat[3], Elma Zaganjor[1,2], Yin Pun Hung[1], Isaac S. Harris[1], Seung Min Jeong[1,2], Lydia W.S. Finley[1,2,b], Gaudenz Danuser[1,c], Sandra S. McAllister[3,4,5,6], Joan S. Brugge[1], Marcia C. Haigis[1,2]**
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Metastatic breast cancer remains a leading cause of cancer mortality in women worldwide, yet our understanding of the metastatic process remains incomplete. Many pro-tumorigenic and pro-metastatic signaling cascades are strengthened by reactive oxygen species (ROS), which are often normal byproducts of cellular and mitochondrial metabolism. SIRT3 is a mitochondrial deacetylase shown to inhibit tumorigenesis through its antioxidant activities. Here, we report that SIRT3 inhibits cell migration and attenuates pro-metastatic signaling by decreasing ROS levels. Furthermore, clinical data suggest that the loss of SIRT3 expression in primary breast cancers leads to increased metastatic frequency. Our results identify SIRT3 as a novel suppressor of breast cancer metastasis and elucidate a new intersection between metabolism and cancer progression.

HHMI-5

Characterization of Neurons Expressing Delta and mu Opioid Receptors in Descending Pain Control

***Sarah A. Low*[1], Brigitte L. Kieffer[2], Allan I. Basbaum[3], Grégory Scherrer[1]**
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The rostral ventromedial medulla (RVM) is a region in the brainstem that gates descending pain control. Reticulospinal neurons in the RVM modulate pain by intercepting incoming sensory information as it enters the spinal cord. Three populations of reticulospinal neurons (ON, OFF, and Neutral cells) have been described based on their firing patterns in response to painful stimuli such that ON cell activity increases and OFF cell activity decreases, while Neutral cell activity does not change. Previous studies have suggested that microinjection of morphine in the RVM is analgesic due to direct inhibition of ON cells and indirect activation of OFF cells. Unlike MORs, however, the role of DORs in the RVM remains unclear. In this study, we used knock-in reporter mice that express DOReGFP and MORmCherry fusion proteins to reveal DOR expressing neurons in the RVM and to examine possible DOR and MOR co-expression. Our results suggest that MOR may not be only

expressed by ON cells and that DOR and MOR are co-expressed in a subset of GABAergic reticulospinal neurons. In combination with ongoing functional studies, our results will elucidate how DORs and MORs cooperate to fine-tune descending pain control.

HHMI-6

Identifying Metabolic Heterogeneity in Head and Neck Squamous Cell Carcinoma Xenografts and Human Tumors

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Fluctuating intratumoral oxygen levels may produce metabolic heterogeneity as portions of a tumor employ aerobic or anaerobic metabolism. Intratumoral metabolic heterogeneity may increase the chances of treatment failure due to the presence of a subset of metabolically flexible tumor cells. Using a head and neck squamous cell carcinoma (HNSCC) xenograft model and real-time imaging, we investigated whether HNSCC develops intratumoral metabolic heterogeneity. After confirming Cal33 cells' altered metabolism in response to hypoxia, xenograft tumors were grown in nude mice. Tumor uptake of fluorescent markers identifying hypoxia, glucose import, or vascularity was imaged simultaneously using fluorescent molecular tomography. The variability of intratumoral 2-deoxyglucose (IR800-2-DG) concentration was used to assess tumor metabolic heterogeneity. IR800-2-DG uptake in hypoxic regions of Cal33 tumors was 2.04 times higher compared to the whole tumor ($p = 0.0001$). Tumors with hypoxic regions had greater metabolic heterogeneity than tumors lacking a hypoxic signal. To translate our findings to clinical care, human HNSCC tumors were assessed for the intratumoral variability of ^{18}F-fluorodeoxyglucose (^{18}F-FDG) uptake in clinical PET-CT scans. ^{18}F-FDG PET-CT scans may be used to stratify tumors according to their intratumoral metabolic heterogeneity, which could be an indicator of prognosis.

HHMI-7

Cranial Nerve Development in Duane Retraction Syndrome

***Jong G. Park*[1,2,3,4,7], Alicia A. Nugent[2,3,4,5], Caroline Andrews[2,3], Wai-Man Chan[2,3], Michelle M. Delisle[2,3], Max A. Tischfield[2,3], Elizabeth C. Engle[1,2,3,4,5,6,8]**

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Duane Retraction Syndrome (DRS) is a congenital cranial nerve dysinnervation disorder, and is characterized by restriction of eye abduction and globe retraction during eye adduction. We previously identified dominant missense mutations in the gene *CHN1* in families with DRS, and we generated a mouse model with a knock-in *Chn1* mutation. We dissected and imaged the orbits of $Chn1^{KI/KI}$ embryos. We determined that the abducens nerve was absent in $Chn1^{KI/KI}$ mice, and the lateral rectus was innervated by an aberrant branch of the oculomotor nerve, recapitulating the human DRS phenotype. We investigated the mechanism of this aberrant innervation by asking if it could occur with an absent abducens nerve but normal oculomotor nerve. To address this, we examined *MafB* knockout mice, which do not develop abducens nuclei or nerves. We dissected and imaged the orbits of $MafB^{KO/KO}$ embryos, and determined that $MafB^{KO/KO}$ mice recapitulate the *Chn1* mice and the human DRS phenotype. Our findings suggest that the aberrant innervation of the lateral rectus by the oculomotor nerve in DRS is secondary to the absence of the abducens nerve. We will further investigate whether this could be caused by axon guidance factors secreted by the extraocular muscles or the surrounding mesenchyme.

Call for Nomination for the George M. Kober Medal and George M. Kober Lecture

This is a Call for Nomination for the George M. Kober Medal for 2017 and George M. Kober Lecture for 2018. He was active in the early days as a leader of several national organizations including the Association of American Physicians—an early organization founded in the 1885 by seven Physicians (including William Osler) an organization which promotes:

> *"the pursuit of medical knowledge, and the advancement through experimentation and discovery of basic and clinical science and their application to clinical medicine..."*

Please provide a brief cover letter highlighting the major accomplishments of the nominee along with an updated CV and submit by December 2, 2015 to **admin@aap-online.org**

George M. Kober Medal

The Kober Medal is awarded annually to a member of the Association of American Physicians upon the recommendation of Council. This award is for those who have contributed to the progress and achievement of the medical sciences or preventive medicine. The first Kober Medal was presented at the Annual Meeting in 1925.

George M. Kober Lecture

The Association of American Physicians honors Kober and continues to honor him by giving their highest award to an honoree(s) every three years to present the Kober Lecture. Those speakers have included at least 13 Nobel laureates to date. This award is for those who have contributed to the progress and achievement of the medical sciences or preventive medicine.

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miR-29: A Molecular Timer that Accelerates Aging

Ayumi Nakamura, **Vijay Swahari, Lisa Kurtz, Jeanette Baran-Gale, Cornelius Flowers, Janakiraman Krishnamurthy, Ned Sharpless, Praveen Sethupathy, Mohanish Deshmukh**
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All across the world, people are living longer than ever before. Consequently, advanced age is a risk factor for many diseases, including hypertension, diabetes, osteoporosis and neurodegeneration. Although research on aging has primarily focused on events that occur beyond the period of development, developmentally-timed events that regulate lifespan remain virtually unknown. MicroRNAs (miRNAs) block gene expression post-transcriptionally, thus regulating hundreds of genes. As aging is a consequence of dysregulation of multiple pathways, miRNAs could promote aging via their ability to regulate a diverse array of genes. We and others have identified miR-29 as a miRNA that is continually induced with age during the entire lifespan of the animal. Interestingly, miR-29 is also induced in mouse models of progeria (premature aging). Conversely, miR-29 was found to be reduced in the long-lived Ames Dwarf mice. These studies show a strong correlation of miR-29 expression in normal, premature and delayed aging. To investigate whether miR-29 could be a molecular timer that regulates lifespan, we generated transgenic mice that overexpress miR-29 under a tetracycline-inducible promoter (miR-29TG). Induction of miR-29 at birth resulted striking signs of premature aging, including alopecia, sclerotic skin, kyphosis, infertility, and reduced lifespan. miR-29TG mice also show a marked increase in senescence-associated (SA)-β-gal staining as seen during normal aging and decreased proliferation in the skin, intestine and spleen. As aging and miR-29 have previously been associated with chronic DNA damage, we examined the possibility that miR-29 overexpression promotes aging via activation of the DNA damage pathway. Indeed, increased pH2AX staining (marker of DNA damage) was evident in miR-29TG mice. In addition, p53-regulated genes associated with senescence were markedly upregulated in both wildtype aged mice and miR-29TG mice. Finally, to critically evaluate the role of p53 in the aging phenotype in miR-29TG mice, we crossed the miR-29TG mice with mice deleted for p53. Strikingly, even a single copy deletion of p53 was sufficient to completely rescue the aging phenotype in miR-29TG mice. Together, our results are the first to show that accelerating the developmental timing of miR-29 induction is sufficient to drive an aging program in vivo by activating the DNA damage pathway. Importantly, the aging phenotype can be rescued with partial deletion of p53. These results indicate that, although miR-29 induces widespread changes in the cellular transcriptome, the outcome of organismal aging is driven by a highly specific pathway. Overall, our results highlight the idea that a single miRNA can act as a developmental timer to regulate lifespan.

Tcfap2c Potentiates Tumorigenesis and Cancer Growth in an Activated Neu Model of Mammary Carcinogenesis

Jung Park[1], **Tong Wu[1], George Woodfield[1], Anthony Cyr[1], James De Andrade[1], Tiandao Li[2], Weizhou Zhang[1], Frederick Domann[1], Ronald Weigel[1]**
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Introduction: TFAP2C and mouse homolog Tcfap2c are transcription factors that influence mammary gland development and regulate the gene expression pattern in luminal and basal breast cancers. While multiple human HER2-positive breast cancer cell lines display genetic dependency on TFAP2C, the role of TFAP2C in HER2-positive breast cancer progression remains poorly understood. **Methods:** MMTV-Neu transgenic mice with and without mammary gland conditional knockout (KO) of Tcfap2c were generated. Latency to palpable tumor formation was compared between control and KO mice. Tumors were characterized by immunohistochemistry (IHC). Paired cell lines with and without Tcfap2c were established through adenovirus-Cre (ad-Cre) infection. Cell viability was assessed by MTT. Transcription target genes of Tcfap2c were established by ChIP-seq and qPCR. **Results:** Mice lacking Tcfap2c demonstrated a significant delay in latency to tumor formation compared to littermate controls that retained Tcfap2c (36 vs 28 weeks, $p<0.003$). Furthermore, all control mice developed tumors while 19% of KO mice failed to develop tumors by 52 weeks ($p<0.04$). Ki67 and cleaved caspase-3 (CC3) IHC showed that proliferation was decreased by 43% ($p<0.05$) while apoptosis was increased 3.3 fold ($p<0.04$) in tumors from KO animals compared to control mice. Athymic mice xenografted with Neu-positive mammary cancer cells lacking Tcfap2c demonstrated a significant delay in tumors reaching 2cm compared to cells that retained Tcfap2c (28 vs 20 days, $p<0.003$). ChIP-seq showed peaks for Tcfap2c in the Egfr promoter region, while ad-Cre mediated excision of Tcfap2c attenuated expression of Egfr. Together, this suggested that Egfr is a direct transcriptional target of Tcfap2c. Moreover, stimulation of Egfr with its ligand Egf increased levels of phosphorylated Erk and cell proliferation compared to nonstimulated cells (24% increase in viability, $p<0.05$). Alternatively, pharmacological inhibition of Egfr reduced levels of phosphorylated Erk and cell proliferation compared to untreated cells (59% decrease in viability, $p<0.05$). **Conclusions:** Tcfap2c potentiates tumorigenesis and cancer progression in HER2-positive breast cancer through the transcriptional regulation of Egfr. These findings have important implications in targeting the Egfr pathway in breast cancer.

Poster Abstracts

1

Mitochondrial CaMKII Heart Overexpression Promotes Atrial Fibrillation (AF) in Mice

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The ROS- and Ca^{2+}-sensing signaling enzyme Ca^{2+}/calmodulin–dependent protein kinase II (CaMKII) plays key physiological and pathophysiological roles within subcellular environments. Mitochondrial-localized CaMKII populations contribute to mitochondrial stress responses, at least during ischemia/reperfusion. We hypothesized that mitochondrial CaMKII activity may drive generalized mitochondrial dysfunction and cardiac pathologies. We developed and validated a mouse that overexpresses CaMKIIdelta targeted to heart mitochondria (mtCaMKII mice). We assessed these novel transgenic animals utilizing in vivo echocardiography, cardiac telemetry, and intracardiac electrophysiological catheterization. mtCaMKII mice have a non-lethal but progressive phenotype of cardiac dilation, which was detectable in mice as young as 1 week of age. The mtCaMKII mice exhibit a particular heart arrhythmia: atrial fibrillation (12/20, 60%) (AF) at a greatly increased rate compared to wild type littermates (1/16, 6.25 %). Increased AF was independent of sex, heart weight or atrial weight. mtCaMKII animals had decreased atrial collagen content compared to wild type animals, suggesting there was no increase in atrial fibrosis caused by mitochondrial CaMKII overexpression. AF in patients and in mice is associated with enhanced Ca^{2+} leak from the type 2 ryanodine receptor (RyR2), which is increased following CaMKII-catalyzed RYR2 post-translational modifications, particularly at the pro-arrhythmic phosphorylation site S2814. We crossed mtCaMKII mice with mice that lacked this phosphorylation site (S2814A knock-in mice) and found that mtCaMKII x S2814A homozygous mice were protected from AF (1/7, 14.2%) whereas mtCaMKII x S2814A heterozygous mice remained arrhythmiogenic (3/4, 75%). In vitro analysis of mtCaMKII cardiomyocytes identified that transgenic overexpression of CaMKII targeted to mitochondria results in increased mitochondrial size, increased mitochondrial ROS (mtROS) production and increased oxygen consumption (OCR) at baseline, suggesting increased mitochondrial stress following mtCaMKII overexpression. Conversely, transgenic mice which overexpress an inhibitory peptide against mitochondrial CaMKII show decreased mtROS and decreased AF following chronic (3 week) angiotensin II infusion (3/9, 33%) compared to the wild type angiotensin II induction rate (9/14, 64%, P=0.06), suggesting that mtCaMKII–governed pathways may have relevance in multiple AF models and potentially other diseases marked by excessive mitochondrial ROS.

2

The Identification of Sphingosine Kinase 2 and Sphingosine-1-Phosphate Receptor 2 as Two Novel and Potent Targets in the Pathway of Epidermal Growth Factor-Induced Cellular Invasion

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Sphingolipids have emerged as bioactive constituents of eukaryotic cells. The bioactive sphingolipid ceramide mediates cellular senescence, apoptosis and cell cycle arrest. Acted upon by ceramidase, it is transformed to sphingosine that in turn gets phosphorylated by sphingosine kinase (SK) to generate sphingosine-1-phosphate (S1P). This lipid, on the contrary, mediates cellular proliferation, mitogenesis, inflammation, angiogenesis, and cancer metastasis through its actions on G-protein coupled receptors (S1PR1-5). ERM (ezrin, radixin, and moesin) proteins are a group of adaptor molecules linking the cortical actin cytoskeleton to the plasma membrane, and are emerging as critical regulators of cancer metastasis and progression via regulation of cell morphology and motility. Recently, our lab has identified S1P as an acute and potent ERM activator (via phosphorylation) through its action on its receptor S1PR2. We have also demonstrated that S1P-mediated filopodia formation, a first step in cell invasion, is through ERM activation. Growth factors are known activators of ERM proteins; however, it is not known if this involves the newly related S1P/S1PR2 axis as well as upstream metabolites of the sphingolipid pathway. Using pharmacological inhibitors, siRNA technology as well as genetic approaches, we have demonstrated that SK2 is not only essential but also sufficient in EGF-mediated ERM phosphorylation. Surprisingly, and for the first time, we proved that this event, although dependent on S1PR2 activation, does not require extracellular S1P secretion. We have also unveiled new mechanisms of SK2 regulation by ErbB1 (EGFR), where these two proteins are in close proximity. Finally, we identified SK2 and S1PR2 as two novel and potent targets in the pathway of EGF-driven invasion. In fact, the inhibition of SK2 or S1PR2 eradicated EGF-mediated lamellipodia formation, and subsequent adhesion and extracellular matrix invasion. We also showed that SK2 overexpression increases EGF-mediated adhesion and invasion; these biologies are abolished upon the overexpression of Ezrin T567A, a dominant negative form of ezrin. In conclusion, this body of work does not only uncover new mechanistic insights for EGF-mediated invasion, it also set the stage for two novel alternative therapeutic targets that could be of utmost importance especially in patients that become resistant to current EGFR-tyrosine kinase inhibitors.

Poster Abstracts

3

Suppression of House Dust Mite-Induced Allergic Airway Disease is Associated with the Appearance of Pro-Regulatory Gut Microorganisms and Increases in Pulmonary Regulatory T Cells

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Introduction: Allergic asthma is a major global cause of morbidity, impacting over 300 million individuals. Asthma is characterized by an inappropriate immune response to harmless allergens such as house dust mite (HDM). Determining how the immune system loses tolerance to allergens is critical for development of curative asthma therapies. Many reports have suggested that decreased regulatory T cells (Tregs) can predispose individuals to developing asthma, and mounting evidence points to the microbiome as a crucial player in Treg induction. **Methods:** To explore the role of the microbiome in immune regulation, allergic airway disease (AAD) was induced in C57BL/6 mice (n=5-9 per group) by daily intranasal challenge with HDM extract for up to 11 weeks. Controls received intranasal saline. Fecal pellets were collected weekly during the exposure period. DNA was extracted from fecal pellets and the V4 region of the 16S rRNA gene was amplified. Amplified DNA was sequenced and processed using QIIME software. At 2, 5, and 11 weeks, groups of mice were sacrificed and cells were isolated from the lungs, broncho-alveolar lavage (BAL), and lung-draining (hilar) lymph node (HLN) for flow cytometric analysis and BAL cellular differentials. Comparisons were made using one- and two-way ANOVA and student's t-test. **Results:** HDM induced hallmarks of AAD with 2-5 weeks of exposure, including BAL eosinophilia and leukocytosis in all lung compartments. By 11 weeks of exposure, tolerance and subsequent disease suppression (i.e. resolution of BAL eosinophilia) developed. This phenotype was associated with an increase in the proportion of Tregs in the HLN relative to control and acute HDM-exposed animals. In addition, distinct changes were noted in the gut microbiome with 11 weeks of HDM exposure. Organisms of the genus Blautia increased from zero to between 5 and 10 percent of all organisms present in HDM-exposed mice between 5 and 11 weeks while remaining undetectable in controls ($p < 0.0001$). A similar pattern was noted for genus Bacteroides, which increased to between 5 and 20 percent of all organisms by 11 weeks in HDM-exposed mice while remaining below 2 percent in controls ($p < 0.0001$). Intriguingly, members of both groups of organisms have been shown to influence the induction and maintenance of Tregs. **Conclusions:** These data demonstrate a correlation between pro-regulatory microbes in the gut microbiome and disease suppression, which suggests that the microbiome may play a role in the regulation of immune responses to inhaled allergens. This work was supported by NIAID (R01 AI-043573 to RST) and NHLBI (F30 HL-126324 to AJA).

4

Hippocampal Pathology in Dementia with Lewy Bodies is Distal to Mossy Fiber Projections in the CA2 Subregion

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As the center of learning and memory in the brain, the hippocampus has been extensively studied in health and disease. Damage to this structure can cause dementia, and it is one of the regions most prominently affected in neurodegenerative disorders such as Alzheimer's disease (AD), but also to some extent in late stage Parkinson's disease (PD). PD is generally considered to be a movement disorder, but a majority of patients eventually experience dementia during the course of the disease, with these cognitive impairments usually quite distinct from those seen in AD. Moreover, some patients develop dementia before the onset of motor symptoms, in which case they are considered to have Dementia with Lewy Bodies (DLB). Despite considerable effort, however, the anatomic and physiologic correlates of dementia in PD and DLB remain unclear. As the diagnosis of dementia in late PD is often complicated by other neuropathology such as concurrent AD, a rigorous examination of the brains of patients with DLB, who are generally younger, provide the best chance to study this form of dementia. Importantly, Lewy bodies and Lewy neurites, which are aggregates of the protein alpha-synuclein, are found to localize to the CA2 subregion of the hippocampus in DLB, just distal to the mossy fiber projections from the dentate gyrus. One goal of this study, therefore, is to generate a gene expression signature for each hippocampal subfield of the human brain, thereby creating a molecular atlas for this important brain structure. This will help in understanding, among other things, what distinguishes CA2 from other regions of the hippocampus. Preliminary studies confirm that the pathology localizes to CA2, but at the same time have raised interesting questions concerning the subcellular localization of Lewy pathology, and what circuitry is involved. Generation of hippocampal neurons from patient-derived stem cells will allow for the study of the physiological mechanisms underlying the disease. Results from these studies will clarify the role of hippocampal Lewy pathology in DLB and late stage PD, and pave the way toward developing more effective treatments for diseases affecting this crucial brain region.

Poster Abstracts

5

A Rich Club Organization in Spinal Cord Injury Interactome Provides Insight into Pathophysiological Mechanisms and Potential Therapeutic Interventions

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Traumatic injury to the spinal cord is associated with multiple neurodegenerative and neuro-inflammatory mechanisms that determine the overall functional outcome of the patients. Additional research is still required to elucidate the complex interactions among the pathophysiological events triggered by spinal cord injury. Progress in understanding the dynamics and inter-dependencies of these pathological processes is key for successful therapeutic interventions. In this work, we used a combined approach of literature mining, natural language processing and network analysis to curate and analyze a spinal cord injury interactome. We have utilized a previously developed in-house tool to mine the scientific literature for genes and proteins with altered expression after spinal cord injury. Interactions among those genes and proteins were retrieved from protein-protein interaction databases and then used to construct spinal cord injury interactome formed of 704 proteins with more than 14,000 interactions. Functional analysis of the interactome revealed that the most enriched pathways are neuronal growth factor signaling, apoptosis, and cytokine signaling pathways. Network analysis of the interactome revealed a rich-club organization formed by a densely interconnected sub-network of hub proteins with the highest impact on the topology of the network. Major regulators of cell growth and apoptosis including FASL (FAS-Ligand), mTOR (mammalian target of rapamycin), MAPK3 (mitogen-activated protein kinase 3), and CDKN1A (cyclin-dependent kinase inhibitor 1A) proteins are the central players in the discovered rich-club. This indicate that an altered balance growth inhibitory rather than growth promoting factors is a key component of spinal cord injury pathophysiology. Furthermore, analysis of drug-protein interactions for drugs with enriched targets in the network revealed that glucocorticoids are among the top hits. Interestingly, glucocorticoids are found to preferentially target the network's rich-club leading to neuro-protective effects through altering the balance within the rich-club toward an anti-inflammatory and pro-survival response. Finally, the curation of the spinal cord injury interactome as well as the discovery of the rich-club provides an avenue for a better understanding of the pathophysiology of the disease and offers researchers an in-silico approach to test the potential effects of candidate therapeutics.

6

Superoxide Induces Pathogenic Neutrophil Extracellular Traps after Liver Ischemia-Reperfusion in a TLR4- and NOX-Dependent Mechanism

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Neutrophil Extracellular Traps (NETs) have recently been described as a novel mechanism by which neutrophils respond to infectious pathogens and sterile alarmins. NETs are extracellular scaffolds composed of nuclear DNA studded with histones and proteins such as myeloperoxidase (MPO) and neutrophil elastase (NE). Stimulation of Toll-like receptors (TLRs) initiates a signaling cascade that includes required activation of NADPH Oxidase (NOX) and Peptidylarginine deiminase 4 (PAD4). The reactive oxygen species superoxide has previously been shown to signal for neutrophil activation and increased proinflammatory cytokine production through TLR4. Neutrophils accumulate in the liver after ischemia-reperfusion (I/R) injury and contribute to inflammation-associated damage. We hypothesize that circulating superoxide generated after liver I/R induces NET formation. WT and TLR4 KO neutrophils were treated with xanthine oxidase and its substrate hypoxanthine to generate extracellular superoxide. We inhibited superoxide generation by allopurinol (AP) and inhibited NOX by diphenylene iodonium (DPI). WT neutrophils exposed to superoxide demonstrated elevated levels of citrullinated Histone H3, a specific NET marker, by western analysis, while AP or DPI co-treatment resulted in only basal cit-H3 expression. In addition, superoxide treatment alone increased free DNA levels as well as MPO-DNA and NE-DNA association. In contrast, TLR4 KO neutrophils expressed only basal cit-H3, free DNA, and protein-DNA associations despite superoxide treatment. Immunofluorescence microscopy revealed superoxide induced characteristic NET fibers produced by WT, but not TLR4 KO neutrophils. We also evaluated intra-hepatic NET formation and tissue injury following hepatic I/R alone or antioxidant pre-treatment with AP and N-acetylcysteine (NAC). MPO-DNA ELISA, a specific assay for NETs, revealed significant NET formation in mice that was reduced by pre-treatment with AP and NAC, correlating with protection from liver injury by histology and serum ALT. Our study demonstrates that non-infectious superoxide induces NETs, and that WT TLR4 and functional NOX are required for this process. During hepatic I/R, NET formation positively correlates with liver inflammation and damage, suggesting that therapies targeting NET formation could minimize liver I/R injury and improve clinical outcomes.

Poster Abstracts

7

Defining Hemogenic Endothelial Cells from Human Pluripotent Stem Cells

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Hemogenic endothelial cells (HE) represent a rare endothelial cell subset that gives rise to hematopoietic stem and progenitor cells (HSPCs) through a process termed endothelial-to-hematopoietic transition (EHT). HSPC development via EHT is a hallmark of definitive hematopoiesis, which is the critical phase for developing the adult hematopoietic system. The underlying mechanisms that regulate human definitive hematopoiesis remain poorly elucidated. This is due in part to inconsistencies in defining HE based on endothelial and hematopoietic surface antigens. Furthermore, prior reports have focused on murine systems where developmental hematopoiesis is biologically distinct relative to humans. Human pluripotent stem cells, including embryonic and induced pluripotent stem cells (hESC/hiPSC) provide well-defined cellular platforms that can be used to study these human mechanisms and can improve the in vitro production of HSPCs potentially suitable for clinical applications. We hypothesized that functional human HE with endothelial and hematopoietic potential could be derived from hESCs. To test this hypothesis, we developed a RUNX1c-tdTomato reporter that could identify and track EHT in hESCs. RUNX1c is a required transcriptional initiator of definitive hematopoiesis and its expression can be utilized in combination with surface antigens to identify putative populations of HE. We found hESCs with stable integration of RUNX1c-tdTom began to express tdTom as early as Day 9 when differentiated from embryoid bodies and exposed to hematopoietic promoting media conditions. By Day 14, $tdTom^+$ cells comprised over 40% of all differentiated cells. $tdTom^+$ cells could be further enriched upwards of 15% when bulk embryoid bodies were sorted for $CD34^+$ hematoendothelial progenitor cells at Day 9 relative to a presorted population. We further defined putative HE populations by sorting $tdTom^+$ hESCs in conjunction with other endothelial (CD31, CD144) and hematopoietic progenitor (CD41a, CD43) surface antigens. $CD31^+CD144^+CD41a^-CD43^-tdTom^+$ cells retained endothelial-like cobblestone morphology and did not generate $tdTom^+$ hematopoietic progenitor cells when cultured in endothelial specific media conditions. However, this population could produce non-adherent $tdTom^+$ hematopoietic progenitors once exposed to hematopoietic media conditions. Furthermore, $CD31^+CD144^+CD41a^-CD43^-tdTom^+$ cells yielded a significant increase in total hematopoietic colony number when cultured in methylcellulose relative to $CD31^+CD144^+CD41a^-CD43^-tdTom^-$ populations, which reflects an enhanced hematopoietic progenitor cell potential. Single-cell RNA sequencing to assess the heterogeneity of putative HE and non-HE populations are ongoing. This will assist in establishing a genetic signature of HE that can be exploited to enhance HSPC differentiation in future studies. Collectively, our data demonstrate HE with the capacity to adopt either endothelial or hematopoietic phenotype can be defined in vitro from human pluripotent stem cells.

8

Analysis of Prostate Localization Culture Data: Should We Review the Significance of Traditional Non-Uropathogens?

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Purpose: Traditional uropathogenic bacteria localize to the prostate in up to 8% of chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) patients and healthy controls. However, reports on the frequency and significance of prostate localization of traditional non-uropathogenic bacteria (i.e. gram-positives excluding E. faecalis) have been highly variable. Here we review prostate localization cultures from multiple diagnoses and analyze localizing bacteria for the ability to induce an inflammatory response. **Materials and Methods:** We retrospectively reviewed prostate localization cultures and the associated patient clinical information from our institution. Cultures were considered to be localized to the prostate if bacteria count was 1 log greater in the EPS or VB3 than that in the VB1 and VB2 (criteria 1), or 1 log greater than only that in the VB2 (criteria 2). Localizing bacteria were then analyzed for their ability to induce an inflammatory response using a monocyte NF-κB expression reporter cell line. **Results:** Using criteria 1, 10.5% of patients had bacteria localize to the prostate and all were gram-positive organisms (89.3% were traditional non-uropathogens). All of these patients had CP/CPPS with a localization rate of 14% when just this subgroup was analyzed. When patients localizing with criteria 2 were added, there were patients seen by other urologists with one of three diagnoses: CP/CPPS, elevated PSA with no pelvic pain, and category II chronic bacterial prostatitis (CBP) or recurrent UTIs. The overall prostate localization rate in this expanded population was 9.1%. Gram-positive bacteria again comprised 100% of group 1 localizations and 92% of group 2. Group 3 was 23.5% gram-negative. While 100% of gram-negative organisms induced a high NFkB response, a subgroup of gram-positive bacteria also induced a high response. There were more pain and voiding symptoms in the high NFkB non-uropathogen group compared to the low NFkB non-uropathogen group, with the pain difference being statistically significant. **Conclusions:** Traditional non-uropathogens are often localized to the prostate but are usually considered to be of no clinical significance. Within this gram positive population, we have found a sub-group that are able induce a strong in vitro inflammatory response and are significantly associated with more patient reported pain. Our data suggests that heterogeneity exists within this population of bacteria that may play both commensal and pathogenic roles in the prostate.

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A Commensal Bacteria-Derived Lipid, Deficient in the Serum of Mutliple Sclerosis Patients, Causes Attenuation of Experimental Autoimmune Encephalomyelitis and an Increase in CD39+ Tregs

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The role of the microbiome in multiple sclerosis (MS) remains unknown. We have identified a Toll-like receptor 2 agonist produced by bacteria of the phylum Bacteroidetes (composing 30% of the normal gastrointestinal flora), called Lipid 654 (L654). We found that L654 can be recovered in healthy human serum and that levels are significantly lower in patients with MS. To determine the relevance of this finding, we administered L654 to mice that were actively immunized to induce experimental autoimmune encephalomyelitis (EAE) and found significant attenuation of disease. The mechanism underlying the attenuation induced by L654 is not yet known. CD39, the rate-limiting ectonucleotidase responsible for the conversion of adenosine triphosphate to adenosine, has been implicated as a critical suppressive mechanism of FoxP3+ T regulatory cells (Tregs) in both EAE and MS. In MS, circulating CD39+ Tregs are decreased and also impaired in their ability to control Th17 proliferation. Intriguingly, CD39+ Tregs are increased during periods of remission in MS patients. The purpose of this study was to 1) determine if L654 could attenuate clinical disease severity in a transfer model of EAE to better test the effect of L654 on activated, encephalitogenic T cells and 2) determine the role of CD39+ Tregs in the disease inhibition caused by L654 administration. An adoptive transfer model of EAE in SJL/J mice was utilized. Mice received transfer of PLP-specific activated T cells and EAE developed 8-11 days later. A 1 ug dose of L654 was administered to mice at day 0 or at day 0 and 7. Mice were observed for disease severity or spinal cords were harvested for cellular analysis. L654 administration significantly attenuated clinical disease severity in the transfer model of EAE ($p < 0.0001$). Cellular analysis of mononuclear cells in the spinal cord at day 18 showed a significant decrease in total cell number ($p = 0.02$) as well as a titrating decrease in percentage of IL-17 producing, IFNγ producing and IL-17/IFNγ double-producing CD4+ T cells in mice administered L654. At day 10, cellular analysis showed a decrease in total mononuclear cell number and an increase in percentage of CD39+ Tregs in mice administered L654. Therefore, L654 is of significant interest as a potential treatment for MS, as it may increase CD39+ Tregs, known to be deficient in MS patients, and potentially suppress clinical disease. Future directions will determine if the suppression induced by L654 is dependent on CD39+ Tregs, and what effect L654 administration has on spontaneous relapses in EAE.

10

Understanding Down Syndrome: Domains of Genome-Wide Gene Expression Dysregulation and Monoallelic Expression in Single Cells

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Trisomy 21 is the most frequent genetic cause of cognitive impairment. To assess the perturbations of gene expression in trisomy 21, and to eliminate the noise of genomic variability, we studied the transcriptome of fetal fibroblasts from a pair of monozygotic twins discordant for trisomy 21. We have shown that the differential expression between the twins is organized in domains along all chromosomes that are either upregulated or downregulated (Nature 2014; PMID 24740065). These gene expression dysregulation domains (GEDDs) can be defined by the expression level of their gene content, and are well conserved in induced pluripotent stem cells derived from the twins' fibroblasts. Comparison of the transcriptome of the Ts65Dn mouse model of Down's syndrome and normal littermate mouse fibroblasts also showed GEDDs along the mouse chromosomes that were syntenic in human. The GEDDs correlate with the lamina-associated (LADs) and replication domains of mammalian cells. The overall position of LADs was not altered in trisomic cells; however, the H3K4me3 profile of the trisomic fibroblasts was modified and accurately followed the GEDD pattern. These results indicate that the nuclear compartments of trisomic cells undergo modifications of the chromatin environment influencing the overall transcriptome, and GEDDs may therefore contribute to some trisomy 21 phenotypes. GEDDs could be the result of genes on chromosome 21, or to the extra chromosomal material. To distinguish between the two possibilities, we use i/ a series of mouse models of human trisomy 21 with different partial trisomies and monosomies; ii/ targeted disruption of one allele of candidate genes in a trisomy background; iii/ fibroblasts from mosaic trisomies 13 and 18. We have also studied the transcriptome (by RNASeq) of more than 350 single cell fibroblasts from the monozygotic twins in order to test the hypothesis that monoallelic expression in trisomy may explain the phenotypic heterogeneity. Approximately 55% of the heterozygote sites of the expressed protein-coding genes showed monoallelic expression; this observation could also contribute to the understanding of the molecular pathophysiology of trisomy 21 and aneuploidies in general. **Acknowledgments:** The members of the Antonarakis laboratory, particularly A Letourneau, F Santoni, G Stamoulis, and C Borel; the members of the J Stamatoyannopoulos, R Guigo, Y Herault, B vanSteensel labs

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Inhibiting Leukocyte Integrin CD11b/CD18 ($\alpha M\beta 2$) with a Novel mAb Salvaged Kidney Function Following Otherwise Irreversible Ischemic Kidney Injury in Cynomolgus Monkeys

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Delayed allograft function resulting from ischemia reperfusion injury (IRI) is associated with increased peritransplant costs and morbidity, as well as inferior long-term survival rates. Leukocyte-mediated tissue injury is a critical checkpoint during the early stages of inflammation in IRI. We have evaluated, in a nonhuman primate renal IRI model, the effects of inhibiting the pro-inflammatory leukocyte integrin CD11b/CD18 on leukocyte-mediated kidney IRI, using a ligand-mimetic inhibitory monoclonal antibody (mAb 107). Crystal structure of mAb107 in complex with the ligand-binding domain of CD111b/CD18 established mAb107 as the first of a new class of "pure" integrin antagonists, i.e. lacking the detrimental "partial agonism" of current ligand-mimetic integrin inhibitors. **Methods:** A 120-minute unilateral renal warm ischemic insult was induced in eight *Cynomolgus* monkeys (wt 2.6-7 Kg) by hilar cross clamping. The contralateral ureter was ligated. Four animals were treated with intravenous mAb 107 (4-12 mg/Kg) and four controls received a comparable volume of saline or irrelevant mAb. Post procedure monitoring included renal function, integrin activation state, and renal biopsies at 2 days, 1 month and at terminal euthanasia (6-9 months). **Results:** All study animals required release of the contralateral ureteral ligature at 2 days post IRI because of the severity of ATN in the ischemic kidney. Subsequent biopsies of the IRI kidney in the 4 control animals revealed progressive tubulointerstitial fibrosis. Re-ligation of the contralateral ureter of the normal kidney 2 days prior to planned euthanasia resulted in a rapid rise in serum creatinine confirming loss of life-sustaining function by the control IRI kidney. In contrast, renal biopsies in the mAb 107-treated animals showed progressive reversal of the early ATN histopathology, and pre-terminal ligation of the contralateral ureter resulted in no or minimal serum creatinine rise. **Conclusion:** Inhibition of CD11b/CD18-mediated leukocyte adhesion at the onset of severe kidney IRI by a pure integrin antagonist alleviated progressive tubulointerstitial renal fibrosis and salvaged kidney function. These findings suggest a potential therapeutic approach for preventing fibroinflammation following IRI, and provide a basis for evaluating the efficacy of this treatment in a renal transplant model.

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Reevaluating the Neuronal Bursting Properties on Potassium Concentration: A Mathematical Modeling Study

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Many neurons, or populations of neurons, in the brain are capable of producing rhythmic bursting activity. This ability is putatively responsible for rhythmogenic functions like breathing and locomotion. *In vivo*, rhythms are generated by synaptically interconnected neuronal networks, whereas rhythmic bursting behavior is often induced *in vitro* by elevating the extracellular potassium concentration ($\mathbf{K_{out}}$). It is known that increasing $\mathbf{K_{out}}$ raises the reversal potentials of potassium and leak currents. However, the complete nature of how these effects underlie bursting activity has yet to be uncovered. A mathematical modeling study was performed to elucidate the interplay between these factors and their roles in a neuron's transition from quiescence to rhythmic bursting. A conductance-based model of a neuron from the pre-Bötzinger Complex (pre-BötC) was used as a basis. A potassium ion component was incorporated into the leak current, and model behaviors were investigated at varying concentrations of $\mathbf{K_{out}}$, taking into account its effect on delayed rectifier potassium current responsible for after-spike hyperpolarization. Model parameters were constrained to match previous experimental findings. The results generated by our model indicate that: (i) *in vitro* bursting behavior with elevated $\mathbf{K_{out}}$ may occur due to attenuation of the delayed rectifier potassium current and (ii) no oscillations are generated at physiological levels of extracellular potassium.

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Withdrawn

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PRKCA Fusion Gene in Congenital Low-Grade Pigment-Synthesizing Melanocytic Neoplasm

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Rare examples of congenital, low-grade, pigment-synthesizing melanocytic tumors (CLGPM) with histologic overlaps with epithelioid blue nevus (EBN) and spitzoid neoplasm have been described but their molecular characteristics and association with either entity have not been elucidated. A majority of EBN (both Carney complex-associated and sporadic) are characterized by the loss of expression of R1α, protein product of the *PRKAR1A tumor suppressor gene whereas spitzoid tumors commonly harbor kinase fusion genes (NTRK1, ROS1, ALK, BRAF, or RET), resulting in activated kinase signaling.* We identified a novel ATP2B4-PRKCA transcript by RNA sequencing in a CLGPM (6-cm scalp mass in a 5-month-old female). The fusion event resulted in

an in-frame linkage of the catalytic domain of the serine/threonine PRKCA (protein kinase C, alpha) with the N-terminal of ATP2B4 and a high level expression of PRKCA protein kinase. The fusion gene transcript was validated by RT-PCR. We then identified another rare CLGPM (1-cm nodule on shin in a 3-year-old male). Break-apart fluorescence in situ hybridization (FISH) for *PRKCA* and *ATP2B4* were designed and applied to *case 2* to reveal *PRKCA but not ATP2B4* gene rearrangement. Microscopically, both lesions consisted of heavily pigmented dermal epithelioid and spindled melanocytes extending into the subcutis. Focal necrosis was present in *case 1*. Sentinel lymph node sampling done in *case 2* showed deposits of melanocytes in nodal capsule/trabeculae. Both patients were alive with no evidence of recurrence at 5-months and 2.5-years of follow-up. By immunohistochemistry, R1α expression was retained in both cases. Array comparative genomic hybridization data showed losses in chromosomes 1p36.33-p35.3, 1q32.1-q44, and 17q11.1-24.2 in *case 1* and a loss in chromosome 9 in *case 2*. Neither tumor had a hot spot mutation in *BRAF*, *NRAS*, *GNAQ*, *GNA11*, *KIT*, *PRKAR1A*, or *TERT* promoter. We identified participation of *PRKCA* in a fusion gene in 2 CLGPMs. *PRKCA* fusion gene has been reported in papillary glioneuronal and lung squamous cell carcinoma. Although kinase fusions are common in spitzoid neoplasms, *PRKCA* fusion gene has never been described in melanocytic neoplasms. *PRKCA* is ubiquitously expressed and has been implicated in various cellular functions including cell proliferation, apoptosis, differentiation, migration, and adhesion. *PRKCA* gene rearrangement might be a defining feature for this rare and poorly characterized melanocytic entity. Break-apart FISH may be helpful in differentiating CLGPM from distinct entities such as EBN and spitzoid neoplasm.

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Microscale Biphasic Extraction of Small Molecules from Multikingdom Cell Culture Models of Infection

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Background: The inflammatory response is an integrated cellular process initiated by the human host after exposure to a viral or microbial challenge. This carefully orchestrated process begins and is tightly regulated by the secretion of oxylipins, which are fatty acid derivatives. Deregulation of the oxylipin response can lead to excessive inflammation, acute lethal inflammatory responses (asthma exacerbations), and diseases such as alveolar broncho-pulmonary aspergillosis (ABPA). Despite their importance in inflammation, oxylipins have not been extensively studied, as it is difficult to extract them from cell cultures for mass-spectrometry analysis. Compounding this challenge, the oxylipin profile of a culture is highly dynamic, requiring the observation of many time points, is strongly dependent on the cells used, requiring an efficient use of rare patient samples, and is greatly influenced by the cellular and chemical microenvironment, requiring physiologically relevant culture platforms. **Methods:** To address these challenges, we developed a microfluidic device, the micrometabolomics platform, which leverages physical principles of surface tension at microscale to generate a passive biphasic system for the extraction of small molecules such as oxylipins. The platform also allows for multi-kingdom cultures; human immune cells, fungi, and bacteria can all be grown in the culture well. Extracted small molecules are then channeled into the existing metabolomics workflow where they are analyzed using LC-MS. Importantly, the micrometabolomics platform supports higher-throughput experiments. This makes it feasible to test multiple time points to better capture the dynamics of chemical signaling. Also, the platform requires little material to operate, an enabling feature when working with expensive reagents and/or rare patient samples. **Results:** Characterization of the micrometabolomics platform demonstrated the importance of extraction solvent selection; principal component analysis of global metabolite profiles clearly distinguishes between samples extracted with chloroform, γ-caprolactone, and pentanol. Additionally, we demonstrated that certain metabolites are produced in proportion to the culture surface area while others are proportionate to culture depth. By culturing *A. fumigatus* on a range of different matrices, including blood, we demonstrated the importance of fungal microenvironment in small molecule production. This characterization informed experiments to assay oxylipin production in co-cultures of *A. fumigatus* spores and human neutrophils or macrophages. Compared to monoculture of each cell type, co-culture of these cells resulted in significant changes in oxylipin production. **Conclusions:** We developed an enabling microculture platform to study fungal-host small molecule interactions with a particular emphasis on oxylipins. Importantly, the micrometabolomics platform requires few cells to operate making it compatible with clinical samples in future studies.

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Probiotics for Clostridium Difficile Infection in Older Adults: Study Protocol for a Double-Blind, Randomized Controlled Trial

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Background *Clostridium difficile* is a pathogen of rapidly increasing public health importance. In 2013, the Centers for Disease Control and Prevention labeled it as one of three "urgent threat" pathogens. An estimated quarter of a million *Clostridium difficile* infections occur in the United States annually, at a resultant cost of 14,000 deaths and 1 billion dollars. *Clostridium difficile* related deaths have risen 400% over the last decade, and current standard antibiotic treatments are only 75 to 85% successful. Besides increasing the risk of antibiotic resistance and side effects, these treatments are very expensive. The most vulnerable population for *Clostridium difficile* is older adults, who make up approximately half of the cases, but account for 90% of the related deaths. Probiotics may have potential as adjunctive therapeutic agents for *Clostridium difficile* infections, however, current data is limited. **Methods** This protocol details a single-site, randomized, placebo-controlled, double-blind, phase two clinical trial. The trial primarily evaluates the effect of four weeks of probiotic therapy on *Clostridium difficile* duration and recurrence. Secondary outcomes include a decrease in fecal cytokines, fecal lactoferrin, and *Clostridium difficile* toxin density in stool, as well as improved functional status. **Results** The PICO trial began in January 2013, and is currently ongoing. As of December 2014, we have enrolled 33 subjects. **Discussion** This pilot study will determine the feasibility and effect size of a larger probiotic intervention randomized controlled trial in patients with *Clostridium difficile* infection. The trial will also address whether probiotics used as adjunctive therapy may ameliorate the symptoms of *Clostridium difficile* infection, especially in older adults. The methods and tools of this protocol have widespread relevance and portability, and have the potential to reduce healthcare associated infections.

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The Role of Endothelial Cell Mineralocorticoid Receptors in Blood Pressure Regulation and Vascular Function

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The vasoreactivity of resistance arteriole beds contributes to blood pressure (BP) by modulating systemic vascular resistance. Beyond BP regulation, the ability of vascular beds to constrict and relax in response to tissue-specific demand is tightly controlled and can be dysregulated in the setting of cardiovascular disease. The mineralocorticoid receptor (MR) is best known as a critical regulator of blood pressure (BP) that functions by responding to the steroid hormone aldosterone in the kidney to modulate sodium retention. Vascular endothelial cells (EC) also express MR and contribute to vasoreactivity by paracrine effects on the underlying vascular smooth muscle cells (SMC). The impact of endothelial MR activation on vasoreactivity remains uncertain with disparate finding reported. Transgenic approaches to clarify the role of EC MR have yielded incongruous findings. A mouse model overexpressing MR in ECs shows increased BP and enhanced vascular contractility, while a mouse model lacking MR in both ECs and leukocytes shows no effect on BP but protection from obesity-induced endothelial dysfunction. To address this controversy, we created a mouse with MR specifically deleted from EC (EC-MR-KO). EC-specific MR deficiency with intact leukocyte MR expression and normal renal MR function was confirmed in this model. Extensive characterization of the BP phenotype by telemetry reveals that EC-MR deletion did not alter systolic, diastolic, circadian, or salt-sensitive BP. The hypertensive response to renin-angiotensin-aldosterone pathway modulators including aldosterone plus salt and angiotensin II were also identical in EC-MR-KO compared to MR-intact littermates. The role of EC-MR in vasoconstriction was explored using wire myography in mesenteric arterioles revealing no difference in constriction to phenylephrine, KCl, endothelin-1, thromboxane agonist, or angiotensin II. However, in the coronary arterioles EC MR deletion resulted in decreased constrictive response to endothelin-1 (49% decrease at a dose of 10^{-8}M, $p < .005$), while the basilar arterioles showed enhanced constriction to angiotensin II in the absence of EC MR (38% increase at a dose of 10^{-7}M, $p < 0.05$). Endothelium-dependent relaxation to acetylcholine and endothelium-independent relaxation to sodium nitroprusside were unaffected by the presence of EC-MR in mesenteric, coronary, or basilar vascular beds from healthy animals. The differential regulation of vasoreactivity among different vascular beds is currently poorly understood; we hypothesize that EC MR participates in tissue-specific pathways of vasoregulation and suggest that improved understanding of these tissue-specific pathways could lead to targeted therapies for various vascular diseases.

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Pre-Operative Video Education has a Positive Impact on Patient Arthroscopic Surgical Experience but may not Improve Satisfaction Scores

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Introduction: Patient education and expectations can affect surgical outcomes almost as much as the treatment itself and effective communication between physicians and patients is critical. To communicate with our patients, surgeons can choose from several different media options. We conducted a randomized controlled trial evaluating the use of a pre-operative video to educate patients undergoing elective joint arthroscopy. We hypothesize that a pre-operative educational video will positively influence patients' surgical experience and result in higher patient satisfaction scores. **Methods:** 267 consecutive patients undergoing elective joint arthroscopy from July 2013 to April 2014 were consented, enrolled in the IRB-approved study protocol and randomized to either a control or experimental group. The experimental group was shown an educational video detailing the surgical experience and also provided with other routine perioperative informational handouts. The control group did not see the video and only received the informational handouts. At the two-week post-operative appointment, all patients completed a patient-reported outcome questionnaire based on validated predecessors. The surgeon and principal investigator was blinded to which group the patients were enrolled. **Results**: Outcome questionnaires for all 267 of the study participants were included in the final analysis. There were no statistically significant differences between the control group and study group for nearly all measures. Satisfaction scores were high in both groups, 4.72 for the experimental group and 4.75 for the control group, out of 5. However, patients who viewed the pre-operative video reported that the routine perioperative informational handouts were less useful than the control patients who did not view the video (p=0.03). **Conclusion**: Both the experimental and control groups reported very high levels of patient satisfaction which may reflect this particular surgeon's other general perioperative communication and education practices and therefore, did not allow us to detect any statistical difference attributable to the use of an educational video. However, patients who were shown the educational video pre-operatively found the routine handouts less helpful than patients who were only given the handouts which may reflect the usefulness of the video and its potential as an effective educational tool for patients. In clinical practices with already high patient satisfaction scores, pre-operative surgical videos may not improve the patients' surgical experience and satisfaction and therefore, may not be worth the extra time and cost to produce. In clinical practices with lower satisfaction scores and other effective communication and educational practices are lacking, educational videos are well received by patients and may be helpful in improving patients' surgical experience.

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Direct Conversion of Adult Rat Cortical Oligodendrocyte Progenitor Cells to Neurons

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The adult mammalian cerebral cortex does not support neurogenesis following injury or disease. Forced expression of select transcription factors has been shown to directly convert terminal differentiated somatic cells to alternate lineages, such as fibroblasts to neurons. Direct conversion of resident glial cells, which far outnumber neurons, through lineage reprogramming may provide an alternative strategy to cell transplantation for neuronal replacement. Oligodendrocyte Progenitor Cells (OPCs) comprise the largest proliferating population in the adult brain. OPCs actively maintain uniform distribution throughout the CNS and rapidly proliferate and migrate to sites of injury. In the current study, various neurogenic transcription factors (Neurogenin2 (NGN2), Ascl1, Sox2, Pax6, Dlx2, and Olig2VP16) expressed during development are screened on cultured OPCs derived from the adult rat cerebral cortex for neuronal conversion. Delivery of the single factor NGN2, converted cultured OPCs to beta-III-tubulin, MAP2, and NeuN expressing neurons with functional electrophysiological properties. The combination of Ascl1 and Dlx2 converted cultured cells to GABAergic neurons. Direct in vivo delivery of retroviral NGN2 to proliferating glia of the adult rat cortex generated immature doublecortin expressing neurons at week post injection (wpi) and NeuN expressing neurons with mature morphology at three wpi. Our results demonstrate that adult cortical OPCs can be directly converted to neurons and may provide a potential avenue for cortical repair.

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NALP7 Protein Expression is Regulated by Ubiquitination in Response to LPS in Human Monocytes

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Introduction: Activation of the innate immune system in response to pathogen associated molecular patterns (PAMPs) is important in the pathogenesis of the acute respiratory distress syndrome (ARDS). Inflammasomes are multi-protein cytoplasmic complexes made up of a pattern recognition receptor (PRR) in the form of a NOD-like receptor (NLRPs or NALPs) coupled with an inflammatory caspase and an adaptor molecule, called ASC. There are a variety of different NALPs that respond to different stimuli. When activated, inflammasomes mediate cleavage of pro-IL-1β into its mature and biologically active form. **Rationale:** While the NALP1 and NALP3 inflammasomes are well described, the NALP7 inflammasome is not as well studied. Elucidating mechanisms of activation and molecular regulation of the NALP7 inflammasome may provide novel therapeutic strategies in ARDS and other inflammatory conditions. **Methods:** Cell culture and treatment, Western blotting, co-immunoprecipitation, confocal microscopy, and recombinant genetics. **Results:** In human monocytes, NALP7 expression is increased in response to treatment with lipopolysaccharide (LPS) in a dose and time dependent fashion. Previously, only acylated lipoproteins (PAM3CSK4) had been shown as a stimulus for NALP7. Under native conditions, NALP7 is degraded over the course of hours, and its degradation occurs within the lysosome. We observe that NALP7 localization to the lysosomes is decreased in the context of LPS administration, and that NALP7 ubiquitination is accordingly decreased in response to LPS. To investigate if NALP7 stabilization is mediated by changes in the ubiquitination balance maintained between E3 ligases and deubiquitinating enzymes (DUBs), we screened several pharmacological inhibitors of DUB activity. We find that NALP7 is decreased in cells treated with 1,10-phenanthroline (a zinc chelator and inhibitor of JAMM family member DUB enzymes) in a dose dependent fashion, but not by other inhibitors. Guided by the inhibitor data, we screened several candidate DUBs in an over-expression model and have identified a DUB that increases NALP7 protein levels. Experiments are ongoing to further characterize this interaction. **Conclusions:** These findings suggest a unique molecular model for endotoxin activation of the inflammasome. Here, deubiquitinase activity of a JAMM family member DUB enzyme is upregulated by LPS to prevent NALP7 degradation thereby activating the inflammasome in response to infection. Further characterization of this pathway and its regulatory mechanisms may lead to identification of novel therapeutic targets for systemic inflammatory disease.

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Higher Referrals for Diabetes Education in a Patient-Centered Medical Home Model of Care

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Purpose: Prior research reports have described low referral rates for diabetes education from primary practices, despite evidence about the effectiveness of services from diabetes educators and nutritionists. The purpose of this study was to compare referrals for diabetes education among patients receiving care from medical home model versus a traditional family practice. **Methods:** Using a de-identified registry extracted from the electronic health record of a university-affiliated medical group, all patients (age 15 to 96) with diagnosis of pre-diabetes or diabetes seen by a family medicine physician at least twice during 2011-2013 were selected for inclusion. During this period, the medical group operated a medical home model for a portion of its patients; medical home members were university employees, spouses or dependents. Medical home patients were compared to the standard university-affiliated family medicine practice according to standard diabetes care indicators. The crude odds ratio for referral to diabetes education (i.e., diabetes educator or nutritionist) was calculated and subjected to multivariate analysis based on clinical measures. **Results:** The registry included 156 patients with pre-diabetes or diabetes who were members of the medical home and 807 patients who received care from a standard university-affiliated family medicine practice. The medical home group was significantly different ($p < 0.05$) in a few ways: age (younger by average of 4.8 years), race (larger percentage of non-whites), service utilization (more classified as high frequency of primary care service utilization), vascular disease (lower prevalence), and obesity (higher prevalence). Approximately 23.7% of included medical home patients had received a referral for diabetes education, which was 10.2 percentage points higher than the standard care group. The crude odds ratio for referral to diabetes education was 1.99 (95% CI: 1.31- 3.03); the adjusted odds ratio for referral to diabetes education was 2.70 (95% CI: 1.68- 4.32). The adjusted odds ratio indicates medical home patients were about 2.7 times more likely to receive a referral when controlling for race, utilization, vascular disease, and A1C control. **Conclusion:** Patients in a medical home model were more likely to receive referrals for diabetes education than patients in a standard university-affiliated family medicine practice. However, the percentage of medical home patients receiving such referrals is remains rather low at 23.7%. This study also indicates there are ample opportunities to improve the referral process in this medical home model where diabetes education services are free to patients and a multi-disciplinary team is available to treat pre-diabetes and diabetes.

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Activity of the Neuroprotective Angiotensin Converting Enzyme 2 is Altered in the Acute Phase of Rat and Human Ischemic Stroke

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Purpose: As stroke is a leading cause of worldwide mortality and morbidity, it is profoundly important to identify biomarkers to aid in early diagnosis and clinical management and discovery of novel and efficacious therapeutic targets. In this study, we assessed the hypothesis that ischemic stroke results in acute changes in rat and human serum activity levels of angiotensin converting enzyme 2 (ACE2), a carboxypeptidase that induces neuroprotective signaling by metabolizing angiotensin II to form angiotensin-(1-7). We further explored the effects of inhibition of endogenous brain ACE2 activity on the severity of stroke in rats, with the intent to provide evidence that may validate ACE2 as a potential stroke biomarker and neuroprotective target. **Methods:** Serum samples were collected from rats that underwent endothelin-1 induced middle cerebral artery occlusion or from ischemic stroke patients and age-matched controls within six hours (h) and again at three days (d) after stroke onset. We assessed enzymatic activity of ACE2 by fluorometric assay. In a separate experiment, we chronically infused an ACE2 inhibitor into the cerebral ventricles of rats for 5d before and 3d after stroke and measured post-stroke neurological function and infarct volume. All animal experiments were approved by the Institutional Animal Care and Use Committee, and were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals.* The collection and use of human serum samples was by written informed consent as approved by the Institutional Review Board of the University of Florida. Data are expressed as mean relative fluorescence units per minute ± SEM. **Results:** Ischemic stroke in rats (n=50) resulted in an initial decrease in serum ACE2 activity at 4h after stroke (621.8±43.04) vs. pre-stroke levels (889.9±67.01), followed by a rebound increase at 3d post-stroke (1051±147.9). These findings were recapitulated in samples from human stroke, where we observed a decreased level of ACE2 activity at ~4h after stroke (153.4±21.48, n=12) as compared to healthy controls (288.4±48.2, n=8), followed by rebound increases at 3d post-stroke (235.9±48.25). Administration of a centrally infused ACE2 inhibitor resulted in significantly worse neurological function at 4h and at 3d post-stroke. **Conclusions:** These data show for the first time a decrease in serum ACE2 activity in the first few hours following an ischemic stroke followed by rebound increases, indicating potential for ACE2 activity as a stroke biomarker. In line with increasing evidence showing a neuroprotective role for ACE2 in stroke, our findings confirm the importance of endogenous brain ACE2 activity in preserving neurological function.

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Internal Carotid Artery Plaque Strain is a Predictor of Brain White Matter Hyperintensity Lesion Burden

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Introduction: Higher internal carotid artery strain has been shown to be a characteristic of unstable carotid plaques that are more likely to embolize. Little is known however, about how these strain measurements correspond with imaging markers of brain health and metrics of brain structure and function. White matter hyperintensities (WMH), which are bright regions seen on T2 weighted brain MRI imaging, are postulated to result from cumulative subclinical vascular injury. Consequently we hypothesized that plaques that are more prone to embolization would be associated with an increased amount of WMH lesion volume. **Methods:** Subjects scheduled to undergo a carotid endarterectomy were recruited from a neurosurgery clinic within the context of an NIH funded study. Prior to surgery, subjects (N=14, male=8, female=6, mean age 69.6 yr) underwent a protocol involving ultrasound strain imaging and 3.0 Tesla MRI of the brain. The ultrasound was conducted with an 11.4 Hz frequency with a single transmit focus set at the depth of the plaque. The MRI involved a 3D T2-weighted fluid attenuated inversion recovery sequence that was acquired in the sagittal plane using the following parameters: TI= 1868 ms; TR = 6000 ms; TE = 123 ms; flip angle = 90°; acquisition matrix = 256 x 256, FOV = 256 mm; slice thickness = 2.0 mm, no gap, yielding a voxel resolution of 1 mm x 1 mm x 2 mm. Regression was used to examine the effect of strain parameters on the end-organ—the brain. **Results:** A linear regression controlling for age and gender found that maximum absolute strain in the surgical side internal carotid artery measured in the axial direction was predictive of WMH burden with a beta value of .595 (t =2.556; p=0.029). For maximum absolute strain measured in the lateral direction, the beta value was 0.719 (t=3.672; p=0.004,). Maximum peak to peak strain, which is the displacement of the plaque between two points of systole in the cardiac cycle, in the axial direction resulted in a beta value of 0.690 (t=3.045; p=0.012). Lastly, for maximum peak to peak strain in the lateral direction, the beta was 0.777 (t=4.856; p=0.001). **Discussion/ Conclusion:** Plaque strain measurement is a novel mechanism to characterize how vascular health affects the brain. In this study, we have shown that higher strain values in plaques of the internal carotid artery are significantly associated with an increased WMH burden, a marker of vascular mediated brain damage.

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Electrical Changes Precede Vascular Changes in the Inner Retina in a High-Fat Diet-Induced Mouse Model of Diabetes

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Purpose: To identify functional and structural deficits of the retina in a high-fat diet-induced mouse model of type 2 diabetes mellitus. **Methods**: We induced diabetes in C57BL/6J mice by Western diet feeding (42% kcal from fat). Metabolic profiles were measured in these animals at 3, 6, and 12 months of age. Vascular defects of the retina were assessed by trypsin digest at 6 and 12 months. Visual deficits were measured at each age using dark-adapted stimulus-evoked electroretinography (ERG). **Results**: Mice on high-fat diet demonstrated significant gains in weight, adiposity, and insulin resistance beginning at 6 months of age. We found no evidence of structural deficits of the retinal vascular network until 12 months of age, when high-fat-fed mice had significantly greater numbers of atrophic capillaries and pericyte ghosts compared to chow-fed controls. Reductions in stimulus-evoked ERG oscillatory potential (OP) amplitudes were present in diabetic mice at 12 months of age, but were not found at earlier ages. However, beginning at 6 months of age, amplitude reductions became highly correlated to degree of insulin resistance. Furthermore, high-fat feeding produced a slight delay in OP implicit times beginning at 6 months and persisted at 12 months of age. **Conclusions**: With prolonged high-fat feeding, mice develop structural and functional deficits of the retina consistent with other animal models of diabetic retinopathy. In high-fat-fed mice, defects in electrical transmission through the inner retina precede detectable lesions in the retinal vasculature and are correlated with degree of insulin resistance. These data suggest that early damage to the neural retina in diabetes may give rise to later vascular disease, the classic hallmark of diabetic retinopathy.

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Regulation of Plasminogen Activator Inhibitor-1 by microRNA-148a: A Novel Target for Antithrombotic Therapy in Obesity

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Thrombotic events such as myocardial infarction, stroke, and venous thromboembolism are a leading cause of mortality worldwide. Obesity is an epidemic in developed countries and is associated with increased incidence of arterial and venous thrombosis. Mechanisms of obesity-induced thrombosis, however, are not elucidated. Lack of mechanistic insight hampers the development of antithrombotic medicines tailored specifically to obese patients. In the current study, we set out to determine whether obesity causes thrombosis via altered microRNA expression. To induce obesity, C57Bl/6J mice were fed a 60% high fat diet. Compared to lean mice, obese mice demonstrated acceleration of carotid artery thrombosis induced by photochemical injury ($P<0.05$) and increased susceptibility to venous thrombosis induced by inferior vena cava ligation ($P<0.05$). Plasma levels of plasminogen activator inhibitor-1 (PAI-1), a potent irreversible inhibitor of fibrinolysis, were elevated in obese mice, suggesting a potential mechanism of thrombosis in obesity. TaqMan low-density microRNA arrays detected downregulation of 41 microRNAs in plasma exosomes from obese mice. Bioinformatic analysis of the down-regulated microRNAs in relation to the PAI-1 3'-UTR identified microRNA-148a as a potential inhibitor of PAI-1 expression in obesity. We confirmed that levels of PAI-1 mRNA were increased, and levels of microRNA-148a decreased, in white adipose tissue from obese mice compared to lean mice ($P<0.05$). Transfection of mouse endothelial cells (MS-1 cell line) with a microRNA-148a mimetic resulted in significant downregulation of PAI-1 expression, demonstrating a causal relationship between microRNA-148a and PAI-1 expression. These data suggest that downregulation of microRNA-148a leads to increased PAI-1 expression in obesity, and that microRNA-148a may be a potential drug target in obesity-induced thrombosis.

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A Computational Model of Antidepressant Effects

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While depression often describes a ubiquitous transient mood state in response to stressful life events, major depressive disorder (MDD, American Psychiatric Association's DSM-IV manual) is a debilitating and severe mental illness that afflicts 16.6% of Americans at some point in their lifetimes. While one in ten Americans is currently taking an antidepressant, the most frequently prescribed antidepressants, selective serotonin reuptake inhibitors (SSRIs), only demonstrate 20%–50% remission rates. Here, we employ a combination of imperative and declarative programming modalities in order to create a functional, computational model of nine principal regions known to interact with the monoaminergic

transmitter systems monsynaptically, in order to elucidate reasons behind the low efficacy rates and ultimately generate hypotheses to increase the effectiveness of pharmacological intervention. We use an imperative program (written in MATLAB) for computationally intensive purposes such as nonlinear parameter optimization using the genetic algorithm, and a declarative specification, written in Maude, for detailed analysis using exhaustive state-space search and state count. Challenging the current view of SSRI action impinging upon autoreceptor desensitization on serotonin-producing neurons, our approach makes each of its nine regions an "agent" in a contract-net system, where agents "negotiate" adjustments in their receptor levels in response to perturbations to ultimately come to an agreement in which the overall balance of transmitter levels is brought back toward normal. Our model effectively recreates acute drug effects collected from the literature, and shows desensitization of key receptors found to be down-regulated in response to antidepressant drugs. We conclude from our model that because a drug that affects one system can affect all systems, combination therapy utilizing the pharmacokinetic properties of multiple drug targets could lead to more effective antidepressant intervention.

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Paracrine Wnt5a Signaling Inhibits the Expansion of Tumor-Initiating Cells via Ryk/TGFbR/Smad2

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The mammary duct comprises well-characterized distinct basal and luminal cell populations. It is largely unknown how paracrine pathways between basal and luminal cell populations participate in tumorigenesis. Previous we found that basal cells containing the mammary stem cells have enhanced tumorigenic capacity compared to the corresponding luminal progenitors in ErbB2-induced mammary tumorigenesis. Transcript profiling of tumors derived from basal and luminal tumor-initiating cells (basal TIC and luminal TIC, respectively) identified loss of Wnt5a, a noncanonical Wnt ligand in basal TIC-derived tumors compared to luminal TIC-derived tumors. Loss of *WNT5A* is correlated with shorter patient survival and promotes pulmonary metastasis in ErbB2-induced breast cancer model. As one of the TGFb substrates, luminal cell-produced Wnt5a induced a feed-forward loop to activate Smad2 in a receptor-like tyrosine kinase (Ryk) - and TGFb receptor (TGFbR)-dependent manner to limit the expansion of basal TIC via a paracrine fashion, a potential explanation for the suppressive effect of Wnt5a in mammary tumorigenesis. The Wnt5a/Ryk module represents a novel mechanism for spatial regulation of TGFb/Smad signaling pathway in the context of mammary gland development and carcinogenesis. It provides the important insight how different epithelial cells can interact within the mammary gland during tumorigenesis and potentially explain the tumor-suppressive effects that normal mammary tissues provide.

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Subcutaneous Allergen-Specific Immunotherapy Attenuates Allergic Inflammation in a Murine Model of House Dust Mite-Induced Asthma

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Background: Allergic asthma is a disorder that leads to widespread global morbidity and mortality. Unfortunately, pharmacologic therapies for asthma are primarily geared towards symptom relief and have limited potential to reduce the overall prevalence of asthma. Allergen-specific immunotherapy (AIT) is currently the only etiologic therapy for allergic disorders and has been shown to alleviate symptoms in patients with asthma. Although the mechanisms by which AIT exerts its effects are actively being investigated, these studies are largely conducted in humans and thus have not examined immunologic changes within the lungs. The goal of this study was to develop a clinically-relevant murine model to specifically investigate the effects of AIT on allergic airway inflammation. **Methods:** IL-10^{gfp} mice were intranasally administered house dust mite (HDM) over an 11-week period to induce allergic airway disease (AAD). Biweekly, subcutaneous AIT with a standardized mite mix extract was initiated following development of early AAD (week 2) and continued for the remaining 9 weeks at either a low dose (10 AU/ml) or high dose (200 AU/ml). Control animals received equal volumes of vehicle (normal saline phenol). Upon sacrifice, bronchoalveolar lavage fluid (BAL) cells were stained with May-Grünwald Giemsa. Hilar lymph node (HLN), lung tissue, and blood cells were stained for flow cytometric analysis. Total serum IgE levels was quantified via ELISA. H&E and PAS-stained lung tissue sections were blindly scored for degree of inflammation and mucus production. **Results:** Both low and high-dose AIT significantly reduced total leukocyte counts ($p < 0.05$) as well as frequency and total number (75% reduction) of eosinophils in the BAL relative to control animals. AIT additionally reduced frequency and numbers of lung Th2 ($CD3^+CD4^+ST2^+$) cells in a dose-dependent manner ($p<0.05$; high-dose AIT vs. control). While serum IgE levels were comparable across groups before initiation of AIT, low- and high-dose AIT prevented further increases in IgE levels (73% and 35% reduction, respectively) relative to control animals. Inflammation scores were significantly decreased by low- and high-dose AIT ($p<0.05$), with low-dose AIT more greatly affecting inflammation and high-dose AIT more greatly affecting mucus production. AIT did not alter levels of local (HLN, lungs) or systemic (blood) $Foxp3^+$ regulatory T cells, which are often elevated in patients receiving long-term AIT. **Conclusion:** Results from this study demonstrate that AIT attenuates severity of AAD by affecting multiple parameters of allergic inflammation. This model may serve as a preclinical tool to further elucidate the mechanisms by which AIT benefits asthmatic patients.

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Poster Abstracts

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Partial Covariance Based Functional Connectivity Computation Using Covariance Matrix Conditioning

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Functional connectivity refers to shared signals among brain regions and is typically assessed in a task free state. Functional connectivity commonly is quantified between signal pairs using Pearson correlation. However, resting-state fMRI is a multivariate process exhibiting a complicated covariance structure. Partial covariance assesses the unique variance shared between two brain regions excluding any widely shared variance, hence is appropriate for the analysis of multivariate datasets as exemplified by fMRI. However, calculation of partial covariance requires inversion of the covariance matrix, which, in most functional connectivity studies, is not invertible owing to rank deficiency. Extant approaches for circumventing this problem include restricting the numbers of brain regions under consideration, employing strong model assumptions, or performing extensive simulations. Here we demonstrate a mathematically simple approach to inverting the high dimensional BOLD covariance matrix. This method employs only weak model assumptions, requires no simulations, improves test-retest reliability of functional connectivity estimates, and is nearly invariant to the use of global signal regression. Application of this technique to a dataset acquired in healthy young adults revealed that resting-state networks are more distinct after removal of widely shared variance. Interestingly, the distinction between two classically opposed networks (the default-mode and dorsal attention networks) and integration of two related networks (the control and salience networks) both were enhanced by partial covariance analysis.

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An Activation Likelihood Estimation Meta-Analysis for the Neurobiological Determinants of Human Descriptive Moral Decision Making

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Introduction: Moral decision making encompasses a wide range of decision types that are paramount to successfully navigating social behaviors with short- and long-term life consequences. Yet the precise neurobiological mechanisms regulating such judgments remain unclear. However, several critical brain structures—such as the amygdala, ventromedial prefrontal cortex, dorsolateral prefrontal cortex, posterior cingulate cortex, medial prefrontal cortex, and the ventral striatum—are implicated in a variety of studies about ethical decision making. Here, activation likelihood estimation is used to conduct a meta-analysis of human neuroimaging experiments reporting areas of brain activation in ethically loaded circumstances. It is expected that these data will help to elucidate the neurobiological basis of human descriptive moral decision making. While the *concepts* of "emotion" and "reason" are often stated as being foundational to human moral judgment and are separated fairly easily in theory, it is possible that such delineated categories are not reflected in neurobiological terms. **Methods:** To identify studies, a literature search using Pubmed and PsycINFO was employed. All studies subsequently underwent a selection process that consisted of reading the articles' abstracts and methods sections. The following exclusion criteria then were applied: (1) non-human studies; (2) amoral studies (original research whose setup did not obviously involve decision making about moral matters); (3) studies focusing on moral sentiments (empathy, sympathy, trust, guilt, disgust, honesty, dishonesty), not decision making itself; (4) decision making about social groups; and (5) moral-decision-making studies whose stimuli did not involve moral dilemmas. **Results & Conclusions:** Data are pending.

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β1-Integrin and BMP Pathway Interactions in the Regulation of Adult Neurogenesis

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The continuous generation of new neurons occurs throughout life in two localized regions of the adult mammalian brain, the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus (DG) of the hippocampus. Ongoing hippocampal neurogenesis has been shown to play a key role in cognition and mood regulation. Diverse signaling molecules present in the SGZ regulate multiple aspects of the neurogenic process, including neural stem cell (NSC) maintenance, proliferation, and differentiation. In particular, inhibition of bone morphogenetic protein (BMP) signaling promotes hippocampal neurogenesis and improves cognition. Recent data suggests that BMP signaling may be modified by interaction with β1-integrin, an extracellular matrix-interacting protein that has been implicated in maintenance of NSCs. We used the Cre/LoxP system to genetically ablate β1-integrin in the dentate gyrus of adult β1-integrin floxed mice or from cultured NSCs derived from the SVZ of these mice. We also performed co-immunoprecipitation studies to investigate the interaction between β1-integrin and the BMP receptor subunits. Our results show that genetic ablation of β1-integrin in NSCs *in vitro* leads to a decrease in levels of stem and progenitor cell proliferation, as well as an increase in astrocytic differentiation, an effect which can be reversed by the addition of the BMP signaling inhibitor noggin. Genetic ablation of β1-integrin in NSCs leads to an increase in overall levels of BMP signaling and β1-integrin physically interacts with the type I BMP receptors in both the wild type adult hippocampus and in cultured NSCs. Conditional knockout of β1-integrin in the dentate gyrus of adult mice alters the size of the NSC pool and modulates cell fate commitment. We conclude that β1-integrin inhibits BMP signaling in adult NSCs and that β1-integrin is critical for adult NSC maintenance and prevention of astrocytic differentiation, thereby allowing for ongoing neurogenesis.

Poster Abstracts

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White Matter Microstructure Contributes to Age-Related Changes in Default-Mode Network Deactivation

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The default mode network (DMN) is a set of intrinsically connected regions that are most active when not specifically engaged in an externally-directed cognitive task. The DMN may play a role in Alzheimer's disease pathology, and it has become an important area of study in neurocognitive aging. Decreases in fMRI BOLD signal during cognitive tasks, termed "task-induced deactivation", are thought to indicate a reduction in metabolic demand of these regions during tasks. Older adults tend to show lower task-induced deactivation in the DMN compared to younger adults. However, little is known about mechanisms underlying this decrease in DMN function. In the present study, we examined whether white matter integrity in tracts connecting DMN regions contribute to age-related changes in DMN deactivation. 53 younger (25-40 years old) and 57 older (60-78 years old) adults participated in an fMRI task-switching experiment and underwent diffusion tensor imaging. Regions of interest (ROIs) were generated on cortical structures showing peak task-induced deactivations across the participant sample. Percent signal change was extracted across all fMRI ROIs to estimate overall deactivation magnitudes in each group. These fMRI ROIs were also used as cortical seed points for structural white matter tractography. Fractional anisotropy (FA) was then computed across all tracts found to connect the ROIs in the tractography analysis. Results from multiple regression analyses revealed correlations between age and DMN task-induced deactivation (r = -0.26, N = 110, p = .007), DMN task-induced deactivation and FA within tracts connecting the DMN (r = 0.28, N = 110, p = .004), and FA within the DMN and age (r = -0.53, N = 110, p < .001). However, a mediation analysis revealed that the total relationship between age and DMN task-induced deactivation (c = -0.26) was explained by the indirect relationship through FA within the DMN (ab = -0.10, 95% CI [-0.216, -0.006]), rather than by the direct effect of age which was not significant (c' = 0.15, 95% CI [-0.349, 0.056]). These findings suggest that declining white matter integrity may be a structural mechanism underlying an age-related inability to reduce metabolic demand in the DMN during cognitive tasks. This has important implications for Alzheimer's disease, as amyloid release may be triggered by metabolic activity at synapses. Therefore, it may be of interest to investigate how this link between declining white matter microstructure and DMN function relates to amyloid deposition.

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The Role Of Tumor-Derived Tslp In Macrophage-Tumor Biology

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Ordinarily, macrophages play critical roles in host defense against disease, including neoplasia. However, macrophages are 'plastic' and can transition between distinct functional states largely influenced by the inflammatory microenvironment. The mechanisms by which these inflammatory cues govern macrophage functionality are incompletely understood, which could lead to the discovery of novel prognostic or therapeutic targets. While much attention has focused on IL-4 or IL-13 as drivers of M2-like macrophages, a subset thought to hinder antitumor activity, we posit that other factors contribute to the complexity and diversity of macrophage functionality. Recent work has shown that thymic stromal lymphopoietin (TSLP), a hematopoietic cytokine known to induce Th2-like responses in allergy models, has been found to directly impact macrophage function and intensify their M2-like activities in these same disease settings. Therefore, we hypothesized that TSLP plays an important, yet, undefined role in macrophage-tumor biology. To test this notion, we made use of the 4T1 mammary tumor model, previously reported to secrete high levels of TSLP in vitro and known to generate profound myeloproliferative responses in vivo. We demonstrated that 4T1 cells produced high levels of TSLP in vivo, proportional to the magnitude of tumor burden. We also demonstrated that knockdown of TSLP expression in 4T1 cells led to significant decreases in tumor growth and metastasis which correlated with significant increases in survival. We have shown that TSLP can impact the secretion of the chemotactic cytokine CCL17 from macrophages in vitro, which has been shown in various cancer models to attract CCR4-expressing tumor cells to metastatic sites. Consistent with this notion, we found that 4T1 cells, in contrast to its well-recognized non-metastatic isogenic variants, 67NR and 168FARN, express high level of CCR4. Moreover, both isogenic variants also express low levels of TSLP. Altogether, these data suggest that tumor-derived TSLP plays previously unrecognized roles in macrophage-tumor biology and profoundly impacts neoplastic progression in patients, a notion we plan to investigate in breast cancer patients.

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Epigenetics of Chromosome Breakage Sites and Translocations

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Chromosome translocations are genetic hallmarks of most cancer cells. Translocations require the formation of DNA double-strand breaks (DSBs) at two or more genomic loci, followed by the illegitimate joining of broken chromosomal ends through DNA repair. There is increasing evidence that translocations occur at non-random sites in the genome, suggesting that certain regions of the genome are more susceptible to DNA breakage than others. We hypothesize that altered chromatin structure predisposes genomic sites to DNA breakage and translocations. To identify chromatin features that facilitate translocations, we have mapped histone modifications and chromatin structure at translocation-prone regions in anaplastic large cell lymphoma (ALCL) precursor cells. We find enrichment of active histone marks and a decrease in repressive marks near frequent translocation breakpoints. In addition, one of the two breakpoints is enriched in hypersensitive sites based on analysis of DNase-I hypersensitivity. In a complementary computational approach, we have identified altered chromatin features including specific histone marks at common leukemia and lymphoma breakpoints in hematopoietic stem cells. In order to directly test the role of chromatin features in DNA breakage susceptibility, we have developed a protein-DNA tethering system that allows us to create local chromatin domains at pre-defined sites in the genome containing inducible DSB sites *in vivo*. By measuring the amount of DSBs using ligation-mediated PCR, we find that histone modifying enzymes that create active chromatin domains generally increase breakage susceptibility. Finally, we have developed a high-throughput interphase FISH assay to detect low frequency chromosome breaks and translocation events in human cells. Overexpression of histone modifying enzymes that generate open chromatin increases chromosome breakage and translocation frequency in ALCL precursor cells when DNA damage is induced. Taken together, these experiments are providing first insights into the role of chromatin structure in the formation of nonrandom chromosomal breaks and the mechanisms that lead to clinically relevant translocations.

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A New Role for Th17 Cells in Controlling *Trypanosoma cruzi* Infection

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Trypanosoma cruzi is the protozoan parasite that causes Chagas Disease, a potentially fatal illness that can result in severe heart damage and GI pathology. The parasite chronically infects about 8 million people in highly endemic areas of Latin America and 300,000 individuals in the United States. CD8+ T cells are crucial for protective immunity against intracellular pathogens, but T helper cells are also needed to attain optimal protection against *T. cruzi*. Although Th1 cells are believed to be the subset of helper T cells most important for controlling intracellular infections, some research has also hinted at a role for Th17 cells and the Th17 cytokine IL-17A in *T. cruzi* immunity. Interestingly, previous work in our lab showed that Th17 cells conferred better protection against a later *T. cruzi* challenge than Th1 cells when adoptively transferred into RAG KO mice with polyclonal CD8+ T cells. In this project, we found that co-culturing macrophages with Th17 cells or IL-17A alone could protect them from *T. cruzi* infection, demonstrating that Th17 cells and IL-17A exert direct protective effects *in vitro*. However, IL-17A was not able to protect $gp91^{phox}$ KO mice with defective NADPH oxidase, indicating that protection by IL-17A and Th17 cells involves the activation of phagocyte oxidase and generation of reactive oxygen species (ROS). Despite these results, neither neutralization nor overexpression of IL-17A had a significant effect on mice's ability to control *T. cruzi* infection, suggesting that another mechanism underlies the protective effects of Th17 cells *in vivo*. Since both Th1 and Th17 cells need CD8+ T cells for protection, we analyzed the helper effects of Th17 cells and cytokines and found that Th17-produced IL-21 was able to significantly activate and expand CD8+ T cells. In summary, this work has illuminated a new role for Th17 cells in the protective immune response against an intracellular pathogen and identified the basic mechanisms for this protection, broadening possibilities for immunotherapeutic approaches. Future experiments will aim to elucidate the exact function of ROS in protection and use *in vitro*-biased Th17 cells from T-bet KO mice and IL-21R KO mice to better analyze the unique effects of Th17 cells and IL-21.

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Loss of Follistatin-like Protein 1 Causes Spontaneous Emphysema in a Murine Model

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Background: Follistatin-like protein 1 (FSTL-1) is a secreted protein that contributes to inflammation in *in vitro* and *in vivo* models, in part by regulating IL-17 mediated signaling. FSTL-1 is produced in the lung and is critical for normal lung development; germline FSTL-1 knock out mice die immediately upon birth and have multiple developmental defects. The physiologic function of FSTL-1 in the lung is unknown. **Methods:** Global, tamoxifen-inducible FSTL-1 knockout mice were generated by through insertion of *loxP* sites into the *fstl1* locus and then crossed to Tamoxifen inducible CAG-Cre recombinase mice. 8-week-old FSTL-1 $^{fl/fl,\ CAG\text{-}Cre/+}$ mice were treated with vehicle (Wild Type) or Tamoxifen resulting in FSTL-1 conditional knockouts (CKO). Ten weeks later lung tissue was collected for histology, protein and RNA isolation. **Results:** Compared to littermate controls, FSTL-1 CKO mice spontaneously developed large bleb, pan-lobular emphysema. Cytokine assessment shows that FSTL-1 CKO mice have increased *il17a*, *il17f*, *ccl2* and *ccl7* production, as well as IL-17 dependent cytokines *csf3* and *cxcl1*, while other proinflammatory cytokines (*tnf*a, *ifng*, *il13*, *il1*β) were similar. Further, FSTL-1 CKO mice exhibited increased *mmp9* and *mmp12* production in the lung as well as increased protease activity as measured by zymography. **Conclusions:** These findings suggest that, in the lung, FSTL-1 exerts a non-developmental role in lung homeostasis. Specifically, loss of FSTL-1 results in the elicitation of Type 17 cytokines, resultant neutrophil and macrophage chemokine production and the subsequent release of MMP9 and MMP12 and protease activity. Thus loss of FSTL-1 leads to spontaneous emphysema development. This suggests that FSTL-1 may be a proximal contributor to emphysema development and a potential avenue for further investigation.

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Withdrawn

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An Essential Role for the Chromatin Associated Sin3B Protein in Hematopoietic Stem Cell Functions

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Hematopoietic stem cells (HSCs), through their ability to both self-renew and differentiate, supply all mature blood cells throughout the life of the organism. In the adult, cell intrinsic mechanisms as well as the bone marrow niche maintain a delicate balance between HSC quiescence and proliferation. Tipping this balance can have detrimental consequences, including bone marrow failure syndromes or hematologic malignancies. Recently, we demonstrated that Sin3B, a component of the mammalian HDAC1/2 corepressor complex, is required for cell cycle exit in response to various stimuli. We hypothesized that Sin3B, through its ability to promote cell cycle exit, engages specific molecular pathways that are central to HSC functions. Analysis of young (8-12 week old) animals genetically inactivated for Sin3B within the hematopoietic compartment revealed an increase in the frequency and number of HSCs and hematopoietic progenitor cells, but no differences in terminally differentiated lymphoid and myeloid cells compared to littermate controls. Additionally, the HSC and progenitor cell compartment of Sin3B deleted animals were skewed and contained less quiescent LT-HSCs and more proliferative ST-HSCs and MPPs. Through competitive transplantation, we determined that Sin3B deleted HSCs were significantly impaired in their ability to engraft and repopulate all the hematopoietic lineages. Moreover, cells derived from Sin3B deleted HSCs were skewed towards the myeloid lineage, reminiscent of aged HSCs. We determined that Sin3B was not required for HSC differentiation through *in vitro* colony forming assays. Furthermore, there was no difference in the ability of Sin3B deleted HSCs to home to the bone marrow of irradiated hosts compared to littermate controls. Together, these studies suggest a cell autonomous and essential role for Sin3B in maintaining both HSCs numbers and functions. We will further discuss whether deregulated cell cycle exit in Sin3B deleted HSCs contributes to our observed effects. **Funding:** NIH (5R01CA148639, 5R21CA155736, T32CA009161) and Samuel Waxman Cancer Research Foundation.

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The Epigenetic Modifier TET2 Regulates CD8+ T Cell Responses Following Viral Infection

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DNA methylation is one of the major epigenetic mechanisms that controls cellular differentiation. In response to pathogens, naïve $CD8^+$ T cells expand and undergo a well-characterized program of differentiation to form a heterogeneous pool of short-lived antigen-specific effector cells and a smaller population of long-lived memory cells that is able to rapidly respond to antigen re-challenge. In a murine model of acute viral infection (LCMV-Armstrong), antigen-specific $CD8^+$ T cells undergo global remodeling of DNA methylation, suggesting that DNA methylation may direct antigen-specific T cell responses. TET2 is a member of the recently identified Ten-Eleven-Translocation (TET) family, which oxidizes 5-methylcytosine (5mC) in DNA to 5-hydroxymethylcytosine (5hmC) and subsequent intermediates, critical steps in the process of DNA demethylation. How TET2 regulates T cell differentiation and function is unknown. Here we demonstrate TET2 expression is regulated by T cell receptor (TCR) signaling in murine T cells. Furthermore, using a novel flow cytometric assay to measure cellular 5hmC content, we find that TCR stimulation also promotes TET activity with the rapid induction of 5hmC. To identify the role of TET2 in T cell responses, we generated mice deficient in TET2 in the T cell compartment ($TET2^{fl/fl}CD4Cre^+$ mice). Loss of TET2 does not substantially alter thymic or peripheral T cell populations. Following infection with LCMV-Armstrong, $TET2^{fl/fl}CD4Cre^+$ mice displayed similar antigen-specific $CD8^+$ T cell expansion and effector responses compared to control mice. However, TET2-deficient mice exhibited significantly enhanced phenotypic memory $CD8^+$ T cell differentiation. Despite preferential $CD8^+$ T cell memory formation, upon re-challenge $TET2^{fl/fl}CD4Cre^+$ mice had blunted LCMV-specific $CD8^+$ T cell recall responses characterized by diminished proliferation, decreased expression of degranulation markers and altered cytokine production. These data demonstrate that TET2 is critical for the regulation of $CD8^+$ T cell differentiation and function.

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A Decrease in Mitochondrial, but not Cytosolic, Iron Protects Against Cardiac Ischemia-Reperfusion Damage Through a Reduction in ROS

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Introduction: Iron is essential for the activity of several cellular proteins, but excess free iron can cause cellular damage through production of reactive oxygen species (ROS). Iron accumulation in mitochondria, the major site of cellular iron homeostasis, leads to cardiomyopathy. However, it is not known whether a reduction in baseline mitochondrial (as opposed to cytosolic) iron can protect against ischemia-reperfusion (I/R) injury in the heart. **We hypothesized that since mitochondria are the major site of iron homeostasis and that mitochondrial iron can lead to oxidative damage, a reduction in mitochondrial iron at baseline would be sufficient to protect against I/R injury.** **Results:** Transgenic (TG) mice with cardiomyocyte-specific overexpression of the mitochondrial iron export protein ATP-binding cassette (ABC)-B8 had significantly lower mitochondrial iron in the heart than nontransgenic (NTG) littermates at baseline, but their cardiac function and the expression of key antioxidant systems were similar to NTG littermates. In response to I/R, TG mice displayed significantly less apoptosis and lipid peroxidation products and better preserved cardiac function than NTG littermates, suggesting that a reduction in mitochondrial iron protects against I/R injury. To confirm these results, we next took a pharmacological approach to assess the effects of a reduction in mitochondrial vs cytosolic iron on the response to I/R using 2,2-bipyridyl (BPD, a mitochondria-accessible iron chelator) and deferoxamine (DFO, an iron chelator that can only reduce cytosolic iron). Treating rat cardiomyoblast H9C2 cells with BPD but not DFO significantly lowered chelatable mitochondrial iron and protected against H_2O_2 induced cell death, and pretreatment with BPD but not DFO protected mice against I/R injury and reduced ROS production, suggesting that a reduction in baseline mitochondrial, but not cytosolic, iron is sufficient to protect against I/R injury. **Conclusions:** Our findings demonstrate that selective reduction in mitochondrial iron is protective in I/R injury. Thus, targeting mitochondrial iron with selective iron chelators may provide a novel approach for treatment of ischemic heart disease.

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Post Burn Hepatic Damage and IL-6 Production is Produced in a p38 MAPK Dependent Manner in Kupffer Cells

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Alcohol intoxication precedes nearly half of all adult burn injuries in the USA and leads to worsened clinical outcomes. As the major site of alcohol metabolism and toxicity, the liver is also critical to the post-burn response. We recently reported systemic inflammation after intoxication and burn is partly caused by derangements in the gut-liver axis though the cell specific mechanisms remain unknown. To this end, C57/BL6 mice were given ethanol (1.2g/kg) by oral gavage 30 min prior to a 15% total body surface area burn. Antecedent depletion of Kupffer cells (KCs) was achieved with clodronate liposomes (0.5 mg/kg) given intravenously. KCs were isolated and analyzed for p38 MAPK with and without LPS stimulation (100ng/ml) and p38 inhibition (SB203580, 20μM). SB203580 (10 mg/kg) was administered via intraperitoneal injection 30 min post burn. The absence of KCs attenuated hepatic damage as measured by a reduction of 53% in serum alanine aminotransferase (ALT) ($p<0.05$), 44% in serum aspartate aminotransferase (AST) ($p<0.05$), a 37% in hepatic triglycerides ($p<0.05$), as well as 77% in hepatic interleukin-6 (IL-6) mRNA expression ($p<0.05$) compared to intoxicated burned mice receiving control liposomes. KCs isolated from intoxicated burned mice demonstrated a >2-fold ($p<0.05$) and 3-fold ($p<0.05$) elevation of baseline and LPS-stimulated p38 activation, respectively. This corresponded to a 2-fold ($p<0.05$) increase in IL-6 production which was decreased by 80% ($p<0.05$) when p38 was inhibited before LPS stimulation. *In vivo* p38 inhibition decreased post-burn ALT and AST by 37% ($p<0.05$) and 32% ($p<0.05$), respectively, compared to intoxicated and burn injured mice given vehicle controls. Antecedent depletion of KCs or post burn p38 inhibition was associated with attenuated pulmonary inflammation 24 hr after injury as seen by histology. In conclusion, p38 MAPK signaling in KCs drives hepatic damage and pulmonary inflammation when intoxication precedes burn suggesting that therapeutic targeting of p38 MAPK after burn injury should be directed specifically at KCs. This work was supported by NIH R01AA012034 (EJK), F30AA022856 (MMC), T32 AA013527 (EJK), and the Falk Foundation.

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Profiling of Alterations in the Interactome of Serine/Threonine Protein Phosphatase Type-1 in Atrial Fibrillation Patients

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Introduction: Global alterations in protein phosphorylation levels associated with dysregulation of serine/threonine protein phosphatase type-1 (PP1) have been implicated in atrial fibrillation (AF), the most common sustained arrhythmia. However, the phosphorylation status of PP1-targeted proteins in different subcellular compartments is not consistently changed in the same direction, underscoring a lack of understanding in PP1 regulation spatially. Since PP1 is composed of a catalytic subunit (PP1c) and a large set of regulatory (R)-subunits that confer localization and substrate specificity to the holoenzyme, we tested the hypothesis that PP1 is dysregulated at the level of these R-subunits in patients with AF. **Methods:** Cardiac lysates were co-immunoprecipitated with PP1c-antibody, separated by SDS-PAGE, in-gel digested, and analyzed by high-resolution mass spectrometry. Protein identification and label-free quantification were achieved using MaxQuant while bioinformatics analyses were performed using MEME and FIMO. Putative PP1c-interactors were further studied using immunocytochemistry, Western blotting, and co-immunoprecipitation. **Results:** 135 and 78 putative PP1c-interactors were captured respectively from mouse and human cardiac tissues, most of which were previously unreported. Of these, 102 in mouse and 60 in human contained at least one of the 3 common PP1c-docking motifs (RVxF, MyPhoNE, and SILK), representing the most PP1c-interactors ever identified from the heart or any other tissue. Applying this protocol to atrial samples from patients with AF or in sinus rhythm (SR), we found increases in binding between PP1c and PPP1R7 (1.6-fold, P=0.04), CSDA (5.7-fold, P=0.001), and PDE5A (1.8-fold, P=0.05) in AF patients. These and other interactors were further validated using bioinformatics, immunocytochemistry and co-immunoprecipitation. In addition, we showed that PPP1R7 overexpression in HEK293 cells drives redistribution of global PP1c. Finally, Western blotting showed that the upregulated associations in AF cannot be ascribed to changes in global protein expression alone, underscoring the importance of studying PP1 at the level of its interactions with R-subunits and not merely at its global level or activity. **Conclusions:** Altogether, this study 1) provided a simple yet powerful tool for studying the PP1 interactome directly from primary tissue, 2) identified and quantified 135 and 78 PP1c-interactors, most of which were previously unreported, from both mouse and human cardiac tissues, 3) discovered three specific alterations in the PP1-interactome in AF patients which may explain the subcellular heterogeneity in PP1 activity and downstream protein phosphorylation observed in AF, and 4) doubled the current knowledge of the PP1-interactome in the heart, thereby opening the way for new avenues of research to better understand the dysregulation of protein phosphorylation underlying disease pathogenesis.

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Identification and Functional Analysis of Key Genetic Drivers of Cutaneous Squamous Cell Carcinoma (cSCC)

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It is estimated that cancer prevention efforts can reduce cancer incidence by over 50%. Unlike for advanced disease, effective molecularly-driven interventions and risk assessment are not available for damaged normal tissue or preneoplastic lesions. Because we have not identified the key genomic drivers of progression from normal tissue to preneoplastic lesion to invasive cancer. We have addressed this gap by using next generation sequencing to understand the mechanisms that drive cancer progression using cutaneous squamous cell carcinoma (cSCC) as a model. cSCC has the most accessible and clinically well-characterized progression sequence of any human cancer; from a distinct precancerous lesion, the actinic keratosis (AK), to invasive carcinoma. This is an ideal model for establishing a paradigm of molecularly targeted cancer chemoprevention. We have analyzed 10 matched sets of human skin, AK, and cSCC using total RNA, miRNA, and exome sequencing. Using functional pair analysis, we have identified multiple miRNA/mRNA pairs, including miR-181 and its target TGFBR3, as potential drivers of cSCC progression. We hypothesize that upregulation of miR-181a promotes initiation and progression of keratinocyte transformation by targeting TGFBR3. We have confirmed upregulation of miR-181a in human cSCC samples in comparison to a cohort of unmatched normal skin specimens by using qRT-PCR, *in vivo*. Our results show that miR-181a has a significantly higher expression (~8.4 folds) in unmatched cSCCs as compared to normal skin. We have shown that miR-181a overexpression and TGFBR3 knockdown significantly suppresses UV-induced apoptosis (38% and 32%, respectively) in HaCaT keratinocytes. We have shown similar suppression of UV-induced apoptosis in primary normal human epidermal keratinocytes (NHEK). We have ascertained that overexpression of miR-181a or direct knockdown of TGFBR3 by shRNA is sufficient for enhanced anchorage-independent survival of HaCaTs. We have examined the effects of miR-181a overexpression or TGFBR3 knockdown on cellular motility and using a matrigel coated PET membrane we have shown that miR-181a overexpression significantly enhances invasion capacity of HaCaTs. In summary, we show that miR-181a regulates susceptibility to apoptosis as well as cellular adhesion and motility at least in part through TGFBR3. Currently, we lack diagnostic predictors of AK progression to cSCC. We propose that miR-181a levels, correlated with TGFBR3 expression, drive disease progression and can be used as a noninvasive biomarker to both predict AK behavior and prognosis in cSCC pathogenesis. Our project is of paramount importance and has a significant translational endpoint that will allow us to identify and ultimately validate biomarkers for cSCC progression and therapeutic targets for cSCC prevention and treatment.

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Exposure to Particulate Matter Air Pollution Decreases DNA Methylation in Alveolar Macrophages via Oxidant Generation

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Background: Particulate matter (PM) air pollution is a global environmental health problem that causes 7 million premature deaths per year worldwide largely due to increased acute thrombotic cardiovascular events. We discovered that PM induces a prothrombotic state and accelerates vascular thrombosis via mitochondrial reactive oxygen species (ROS)-dependent release of IL-6 from lung macrophages. While we were able to use standard reductionist approach to show that mitochondrial ROS-induced release of IL-6 plays a key role in PM-induced toxicity, our data and those from others suggest that PM alters gene transcription through complex pathways, including epigenetic modifications. Dynamic chemical modifications of DNA by cytosine methylation (5mC) and hydroxymethylation (5hmC) represents a fundamental mechanism of biological regulation; however, it is not known whether PM induces epigenetic changes via mitochondrial ROS. **Objective:** To determine whether PM-induced epigenetic changes are mediated via ROS production. **Methods:** We treated MH-S cells, a murine alveolar macrophage cell line, with PM (10 µg/cm2) or control vehicle (PBS) and measured total 5mC and 5hmC on DNA and total m^6A on RNA in the absence or presence of an antioxidant, EUK-134 (a synthetic superoxide dismutase/catalase mimetic). We also measured enzymes that regulate and maintain 5mC and 5hmC (DNA methyltransferases) and (TET enzymes) using qRT-PCR. **Results:** Compared to control treatment, PM caused a reduction total 5mC and an increase in total 5hmC on DNA. PM treatment also decreased total m^6A. All of these PM-induced changes were attenuated in MH-S cells treated with EUK-134. We found that *dnmt 1* mRNA and *tet 1, tet 2* and *tet 3* mRNA levels were decreased after PM treatment. **Conclusions:** Exposure of alveolar macrophages to particulate matter decreases global DNA methylation and increases DNA hydroxymethylation, which are attenuated with antioxidant therapy. Particulate matter also alters the levels of enzymes that regulate DNA methylation and hydroxymethylation. These results suggest that generation of ROS is required for PM-induced epigenetic changes.

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Near Infrared Optical Imaging Sheds New Light into Visualization and Quantification of Dystrophic and Damaged Muscle Health

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Background: Duchenne muscular dystrophy (DMD) is caused by mutations in the dystrophin gene, ultimately resulting in physiological degeneration and clinical weakness of muscle. Current methods of assessing disease involvement in muscle include biopsy and functional testing, but intrinsic variables such as invasiveness and patient compliance limit these two testing modalities, respectively. One means of assessing the state of disease in DMD includes magnetic resonance (MR), which has been demonstrated to non-invasively detect disease involvement by changes in T_2 relaxation of muscle. In this study, we demonstrate the ability of another novel non-invasive technology, near infrared optical imaging (OI), to sensitively and specifically detect involved muscle in DMD following confirmation of techniques by MR. **Methods:** Hindlimbs of *mdx* mice (6 weeks of age) were first compared to age matched unaffected control mice by MR and OI methods. Furthermore, MR and OI data were collected from older *mdx* mice (12-32 weeks of age) that were subjected to a downhill treadmill running exercise bout to induce further muscle damage. All mice were imaged on an Agilent 4.7T MR Scanner and an IVIS Spectrum In Vivo System to capture MR and OI data, respectively. Multiple slice, spin echo scans were acquired to assess MRI-T_2 relaxation and ^1H_2O spectroscopic relaxometry was assessed using STEAM spectroscopy within the soleus, using NNLS analysis on the spectroscopic data. Prior to OI, mice were intravenously injected with 100 µL of Indocyanine Green, an FDA approved fluorescent contrast agent. 2D fluorescence images were captured using excitation and emission wavelengths of 745 and 820 nm, respectively. **Results:** In *mdx* mice, MRI-T_2, ^1H_2O-T_2, and radiant efficiency were significantly elevated in hind and forelimb muscles compared to unaffected control mice. Additionally, following downhill treadmill running, MRI-T_2, ^1H_2O-T_2, and radiant efficiency increased significantly in the post-treadmill cohort of *mdx* mice. **Conclusion:** MR confirmed disease involvement and OI demonstrated the ability to detect muscle damage in a safe, sensitive, and specific manner, resulting from natural disease progression and muscle damage exacerbated by an exercise routine.

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Branch-Specific Dendritic Ca^{2+} Spikes Induce Persistent Synaptic Plasticity

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The brain has an extraordinary capacity for memory storage, but how it stores new information without disrupting previously-acquired memories remains unknown. We show that different motor learning tasks induce dendritic Ca^{2+} spikes on different apical tuft branches of individual layer V pyramidal neurons in mouse motor cortex. These task-related, branch-specific Ca^{2+} spikes cause long-lasting potentiation of postsynaptic dendritic spines active at the time of spike generation. Notably, when somatostatin-expressing interneurons are inactivated, different motor tasks frequently induce Ca^{2+} spikes on the same branches. On those branches, spines potentiated during one task are depotentiated when they are active seconds prior to Ca^{2+} spikes induced by another task. Concomitantly, increases in neuronal activity and performance after learning one task are disrupted when another task is learned. These findings indicate that dendritic branch-specific generation of Ca^{2+} spikes is critical for establishing long-lasting synaptic plasticity, thereby facilitating information storage associated with different learning experiences.

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Incorporation of an Acid-Cleavable Targeting Ligand to Increase Delivery of Nanoparticles Across the Blood-Brain Barrier

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Delivery of therapeutics to the central nervous system (CNS) is severely limited by the presence of the blood-brain barrier (BBB). Recent work has shown transferrin (Tf)-containing gold nanoparticles (Au NP's) of moderate avidity can enter the CNS from the bloodstream by receptor-mediated transcytosis (RMT). To improve delivery of Au NP's to the CNS, an acid-cleavable moiety designed to break during RMT was incorporated between the targeting ligand and Au NP core. Au NP's containing a cleavable Tf ligand show improved ability to cross an in vitro model of the BBB compared to non-cleavable Tf, non-cleavable high-affinity Tf receptor antibody (mAb) and cleavable mAb-containing particles. In vivo results show greater accumulation of the cleavable-Tf particles within the CNS after systemic administration, suggesting that the presence of the cleavable group increases the ability of Tf-containing GNP's to cross the BBB.

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Treatment of Bladder Cancer Cell Lines with Targeted Therapies Promotes Changes in Bladder Differentiation State

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Background: Bladder cancer is the sixth most prevalent cancer in the U.S. Current chemotherapy regimens for late stage and metastatic disease include cisplatin, gemcitabine and mitomycin C. However, for most patients these therapies are not well tolerated. Despite multiple decades of clinical use, mechanisms of therapeutic resistance are not well understood. One possible mechanism is that unique differentiation states within cancer cells confer drug resistance. Normal bladder epithelium is known to contain at least three cellular differentiation states: basal, intermediate and umbrella, distinguished by unique cytokeratin expression (cytokeratin 5/14, 5 and 20, respectively). In order to determine whether bladder cancers contain heterogeneous cellular differentiation states we examined the expression of cytokeratin 5, 14 and 20 in a panel of bladder cancer cell lines. Staining intensity was quantified both before and after treatment with standard of care chemotherapy agents and with targeted agents. By better understanding this tumor heterogeneity we hope to elucidate novel mechanisms of therapeutic resistance and identify predictors of therapeutic response. **Methods and Results:** The differentiation states of a panel of ten bladder cancer cell lines (Cal29, HT1376, JMSU-1, RT112, SCaBER, TCCSUP, UBLC-1, UMUC9, UMUC10 and VMCUB1) were assessed by immunofluorescence and heterogeneity within the cell lines was determined. Cells were then treated with various concentrations of cisplatin, gemcitabine and mitomycin C, singly or in combination. Cells were also treated with various concentrations of targeted therapies such as neratinib, a dual EGFR/ERBB2 tyrosine kinase inhibitor, BEZ235, a PI3K inhibitor, dovitinib, an FGFR3 inhibitor, GSK343, an EZH2 inhibitor, vorinostat, a histone deacetylase inhibitor and (+)-JQ1, a bromodomain inhibitor. Cells were assessed for expression of cytokeratin 5, 14 and 20 by immunofluorescence after 72 hours and heterogeneity based on expression patterns was ascertained. **Conclusions and Future Directions:** Diverse differentiation profiles were observed across this panel of bladder cancer cell lines. The heterogeneous expression patterns of cytokeratin 5, 14 and 20 in these cell lines varied upon treatment with cisplatin, gemcitabine, mitomycin C, neratinib, BEZ235, dovitinib, GSK343, vorinostat and (+)-JQ1. Targeted therapies promoted distinct changes in differentiation state. These results will be analyzed to determine if these changes correlate with drug sensitivity. Future studies will examine the signaling networks that regulate these differentiation states and drug sensitivity, with the goal of developing better combinatorial therapies and personalizing cancer treatment.

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Colorimetric Detection of SNP Variants Encoding Antibiotic Resistance in Mycobacterium Tuberculosis

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Access to fast and robust molecular diagnostics is critical to the successful management of health conditions. Detection of TB and identification of any drug resistance it may carry is a bottleneck preventing timely treatment. The distribution of TB cases is skewed toward low-income countries with a poor medical infrastructure, where point-of-care (POC) diagnostics are the most economically-feasible detection modality. PCR-based assays are the mainstay tools for drug-resistance genotyping because they are able to amplify small amounts of nucleic acids found in easily obtainable biospecimens such as sputum. However, most existing implementations require advanced instrumentation such as real-time PCR machines to read signals from fluorescent probes. Harnessing PCR's sensitivity while eliminating the prohibitive cost of quantifying its output by fluorescence would pave the way for large-scale use of field-ready diagnostic kits in poor nations. We achieved colorimetric identification of several antibiotic resistance-conferring single nucleotide polymorphisms (SNPs) in Mycobacterium tuberculosis using a PCR- and G-quadruplex-based genotyping assay. The assay relies on a G-quadruplex-generating polymerase chain reaction, where amplification is achieved using target-specific primers that are extended by a 5' sequence complementary to a G-quadruplex deoxyribozyme. The peroxidase activity of the G-quadruplexes is then harnessed to convert the chromogenic substrate 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) from its colorless neutral form to a blue-green radical cation, thereby visually reporting a successful amplification reaction and hence the presence of the SNP allele. G-quadruplex-generating PCR for SNP distinction was validated using artificial DNA templates and genomic DNA extracted from M. tuberculosis H37Rv. Plasmids containing segments of wild type (WT) or SNP M306V of the M. tuberculosis gene embB were used as templates for distinction by G-quadruplex-generating PCR. Peroxidase activity of the accumulated G-quadruplexes was assayed colorimetrically by measuring absorbance in real time using TECAN Safire and by visual examination at end-point. Colorimetric detection of the rpoB–WT and rpoB–Q513L alleles was demonstrated using M. tuberculosis genomic DNA. In conclusion, G-quadruplex PCR can be a sensitive yet visual colorimetric gene detection and SNP distinction platform, enabling scoring without electronic devices.

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11 Things You Didn't Know About HR/LR Rats and the Role of Serotonergic Circuits in Stress Coping Style, Number 7 will Blow Your Mind!

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Stress is one of the most widely studied environmental risk factors for the development of a variety of mental illnesses. Stress coping styles encompass a range of physiological, psychological, and behavioral responses aimed to avoid or tolerate distress, and are broadly characterized as "proactive" (a fight-or-flight response to defeat/escape a stressor) or "reactive" (a withdrawal response to avoid or outlast the stressor). In humans, proactive vs. reactive coping styles convey risk or resilience to psychopathology depending on the type of stress, since each coping style can be adaptive in some circumstances but maladaptive in others. To investigate the neurociricuit and molecular mechanisms underlying different stress coping styles, we utilized selectively bred high-responder (HR) and low-responder (LR) rats. HR/LR rats were selected for on the basis of high and low novelty-induced locomotion, and are a well-developed model for studying individual differences in emotionality and behavior. Here we show that HR rats display a proactive coping style while LRs exhibit a reactive coping style in the defensive burying test. HR rats spend more time burying the probe following a single electric shock (proactive coping), while LR rats spend more time immobile (reactive coping). Dual-labelling immunocytochemistry experiments are identifying cells that express c-Fos, an immediate early gene and marker of neuronal activity, and tryptophan hydroxylase (TPH), a marker of serotonergic neurons, to map patterns of neuronal activation in raphe nuclei in HR versus LR rats following the defensive burying task. This points to a role for the raphe nucleus in regulating stress coping style. Future work will further characterize serotonin circuit differences in proactive and reactive coping animals by examining HR vs. LR expression of microRNAs that regulate serotonin-related genes (such as TPH) to identify potential molecular mechanisms for differences in circuit activation.

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Functional Mitral Regurgitation Fibrotic Remodeling is Repaired by Normalizing Valve Hemodynamics: Investigation Using a Flow Loop Bioreactor

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Introduction: Functional Mitral Regurgitation (FMR) is a mitral valve (MV) disorder that results from distortion of the MV annulus and subvalvular apparatus, leading to tethering and tenting of the leaflets. Previously, we established a 1-week *in vitro* FMR model, resulting in fibrotic remodeling. We are interested in investigating whether reverting FMR disease geometry to control after one week of culture can reverse FMR fibrotic changes. The impact this reversal model has on mechanical and biochemical properties of MVs could inform how FMR treatment impacts valve remodeling. **Methods:** We created a pseudo-physiological flow loop system, where MV annular area and papillary muscle position can be manipulated. FMR was obtained through annular dilation and apical-lateral papillary muscle displacement. Valves are cultured in diseased geometry for one week. After one week, the MV is removed from the flow loop, papillary muscles are repositioned to control geometry, and annular area is reduced to control dimensions. The valves are cultured for an additional week in reversal conditions. After two-week culture, valves are analyzed for mechanical and biochemical properties. Biochemical and histological analysis are performed to detect changes in extracellular matrix proteins, remodeling enzymes and glycosaminoglycans in reversal valves compared to two-week FMR and control valves. **Results:** 2-week FMR tissues exhibit trends of being stiffer, less extensible, and more brittle in comparison to 2-week control tissues. Additionally, reversal models showed a recovering of elastic modulus (FMR +52%, reversal -8% compared to control) and extensibility (FMR -18%, reversal +3% compared to control) towards control valve values. Similar trends were seen in chordae tendineae values for elastic modulus (FMR +103%, reversal +44% compared to control), extensibility (FMR -25%, reversal -6% compared to control), and ultimate strain (FMR -21%, reversal -12% compared to control). **Conclusions:** Previous 1-week FMR model studies showed that valves subjected to FMR conditions undergo fibrotic tissue remodeling, which results in more brittle and stiffer tissue. A similar relationship exists between 2-week control and FMR tissues. Reversal model tissues show a trend of reverting to control properties, as they are less stiff, more extensible, and less brittle than 2-week FMR tissues. This work suggests that the MV is undergoing constant and active remodeling to its hemodynamic environment, and placing the MV in normal flow conditions will result in a healthy valve phenotype even after fibrotic remodeling has taken place. **Disclosures:** Dr. Stephen Little has received research funds from St. Jude Medical, Medtronic Inc, Abbott Structural. Dr. Jane Grande-Allen has served as a consultant for Edwards Lifesciences.

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Localization and Molecular Mechanisms of CFTR in Airway Smooth Muscle Cells

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Typical pulmonary manifestations of cystic fibrosis (CF) include chronic airway infection/inflammation, mucus accumulation, and airway obstruction. In addition, airway hyper-responsiveness has been frequently reported in people with CF. The similarities between CF and asthma suggest that an altered airway smooth muscle (ASM) physiology may contribute to CF airway disease. The aim of this study was to determine if CFTR is expressed in ASM cells and whether lack of CFTR alters the ASM cell phenotype. CFTR mRNA was present in newborn non-CF ASM, but absent in CF ASM tissue. Using tyramide signal amplification, our preliminary immunostaining data suggest that CFTR primarily localizes to the cytoplasmic and perinuclear regions. Following CFTR overexpression in ASM cells with an Ad-CFTR-GFP construct, CFTR exhibited a cytoplasmic predominant staining pattern. The wild-type CFTR overexpression pattern was similar to CFTR-F508del overexpression suggesting that expression may be limited to the endoplasmic reticulum/sarcoplasmic reticulum (SR) complex. To investigate whether CFTR expression was important for Ca2+ handling, we utilized fura-2 staining to visualize cytoplasmic stores of calcium. Following cholinergic stimulation, non-CF and CF ASM cells had similar peak Ca2+ responses, but return to baseline Ca2+ levels was delayed in CF. This study suggests that CFTR is primarily expressed in a subcellular compartment of ASM cells and loss of CFTR may be, in part, responsible for the asthma-like phenotype observed in CF. Supported by American Asthma Foundation and Cystic Fibrosis Foundation.

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Regulation of CTL Trafficking by Tumor Vascular Disruption

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Despite recent advances in cancer immunotherapy, inefficient trafficking of tumor-specific cytotoxic T lymphocytes (CTL) to the tumor yield low numbers of immune cell infiltrates, resulting in a poor clinical response. Poor tumor control associated with immunotherapies may be due to inefficient CTL trafficking and/or CTL exhaustion. Here we demonstrate that the initial accumulation of tumor infiltrating CTL is associated with impaired tumor vasculature and reduced entry of additional CTL into the tumor. We have utilized the B16 mouse melanoma model to demonstrate that adoptive cell transfer of optimally primed tumor-specific CD8+ CTLs resulted in early tumor regression but restricted the number of spleen-derived CTL that arrived in tumors 7 days after the initial CTL influx. Live animal imaging by ultrasound Doppler measurements show that blood flow was reduced beginning ~4-6 days following CTL adoptive transfer. Histological evaluation of PECAM-1 staining patterns at this time point revealed reduced microvascular lumen number and area in CTL treated tumors. Recent studies have indicated that vascular normalization via low dose anti-angiogenesis therapy promotes increased CTL access to tumor. Preliminary studies using the VEGFR2 inhibitor axitinib indicated that axitinib-normalized tumor vasculature was resistant to the CTL associated disruption. Together, these results suggest that the reduction in late phase CTL influx resulting from prior CTL-associated vascular impairment can be overcome by combining pre-treatment and coincident treatment with angiogenesis inhibitors to allow sustained CTL access to tumors.

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Increasing the Temperature Sensitivity of the Live Attenuated Influenza A Vaccine Results in Increased Safety While Maintaining Protective Efficacy

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Influenza A virus is a respiratory pathogen that infects through the upper airway and creates pathogenesis through replication in the lower airway. The temperature gradient between these two areas led to the development of the live attenuated influenza vaccine (LAIV) that can replicate in the cooler upper respiratory tract and trigger a protective immune response, while its replication is limited in the lower respiratory tract making safe for administration. This temperature sensitivity (ts) is imparted by 5 mutations within the viral replicative machinery: namely PB2 N265S; PB1 K391E, D581G and A661T; NP D34G. Though this vaccine has an overall acceptable safety profile, it is not licensed in children under two due to concerns of elevated hospitalizations due to wheezing. For this reason it is also not licensed in asthmatics or the immunocompromised. Therefore creation of vaccines with increased safety over LAIV is desirable. Here we show that a previously described PR8 virus containing the 5 ts loci of LAIV has greatly reduced pathogenicity in mice, but importantly still retains lethality at high dose. We have used this platform to evaluate the safety and protective efficacy of two additional cohorts of mutations that increase temperature sensitivity. These novel vaccines may serve as candidates for creating LAIV with improved safety for universal administration to children aged 0-18.

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Heterozygous PROKR2 Mutations May Cause Isolated GnRh Deficiency via Multiple Mechanisms

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Background: Mutations in *PROKR2*, which encodes Prokineticin Receptor 2, cause Isolated GnRH Deficiency (IGD) in humans. In contrast to other autosomal recessive genes causing IGD, >90% of *PROKR2* "mutations" are heterozygous; they are also typically missense, increasingly present in normative databases, and segregate incompletely within pedigrees. These facts raise the question of whether these are truly causal "mutations"and, if so, how they contribute to IGD. One IGD-associated *PROKR2* mutation has been shown to have dominant negative (DN) activity *in vitro*, offering a potential explanation for how other heterozygous rare sequence variants (RSVs) may cause disease; however, it is unclear if this is a widespread phenomenon. **Goals of This Study:** 1) to comprehensively assess the functional effects of all known RSVs in *PROKR2* 2) to model heterozygosity seen in IGD by examining effects of mutant PROKR2 co-expressed with wild-type (WT). **Methods:** Sixty RSVs (<1% minor allele frequency) were found and all were studied: 14 were found in IGD patients, 31 were reported in control populations, and 15 were seen in both. Mutant (Mt) and WT *PROKR2* were either transfected alone or co-transfected together into HEK293 cells at fixed ratios. *PROKR2* signaling via the mitogen-activated protein kinase (MAPK) pathway was assessed using an *Egr1*-luciferase reporter assay. PROKR2 protein expression and trafficking were also investigated using western blots and confocal microscopy of GFP-tagged PROKR2. **Results:** When transfected alone, 49 Mt proteins showed LOF with 33 having <50% WT maximal activity. When WT was co-transfected, 4 Mt proteins exerted a DN effect. Of note, all 4 of these DN mutations were associated with IGD. Surprisingly, the function of a majority of the remaining Mts, including some associated with IGD, was rescued (i.e. signaling activity restored to WT levels) when co-expressed with WT. Still, other Mt proteins showed reduced signaling, suggesting they were either hypomorphs or caused haploinsufficiency. **Conclusions:** By conducting a comprehensive functional analysis to accurately model the heterozygous context of RSVs in *PROKR2*, we conclude that: 1) In the context of heterozygosity, loss-of-function RSVs in *PROKR2* do not always cause a reduction in MAPK signaling, suggesting that they are insufficient to cause IGD and may represent false positive associations. 2) Only a few *PROKR2* RSVs exhibit dominant negative activity, yet are these are always associated with IGD, suggesting they are true causal "mutations." **Funding:** R01 HD015788; KHC by T32 DK007028; LMBOby CAPES 18279-12-0

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Tackling Anti-Angiogenic Therapy Failure in Pancreatic Ductal Adenocarcinoma

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Pancreatic ductal adenocarcinoma (PDAC) is the 4th leading cause of cancer death in the US with a 5-year survival of 6%. Because angiogenesis, the sprouting of new blood vessels from existing vessels, is a process exploited by many cancers for tumor growth, its importance and therapeutic potential in PDAC have been explored both in pre-clinical and clinical studies. Human PDACs (hPDACs) overexpress a number of angiogenic growth factors and receptors, and anti-angiogenic agents result in reductions in tumor volume, tumor spread, and microvessel density, and lead to improvements in survival in subcutaneous and orthotopic nude mouse models of hPDAC. However, clinical trials using anti-angiogenic therapy have been overwhelmingly unsuccessful. We hypothesize that there are many potential reasons for this, including incorrect patient selection and/or inappropriate pre-clinical models, and we will address each of these reasons separately. Because poorly differentiated hPDAC tumors may be relatively vascular, it was postulated that these patients might selectively benefit from anti-angiogenic therapy. To test this theory, we quantified CD31 staining of endothelial cells from over 50 hPDAC samples and determined that differences in vascularity between these PDAC cases were independent of differentiation status or stromal abundance. We hypothesized that gene expression data could inform PDAC angiogenic status. Accordingly, we analyzed RNA-Seq data from The Cancer Genome Atlas (TCGA) and determined that only a small subset of PDAC patients express a strong pro-angiogenic gene signature, raising the possibility that targeted anti-angiogenic therapy should be limited to this subset of patients. Importantly, most anti-angiogenic pre-clinical studies utilized subcutaneous or orthotopic nude mouse models of hPDAC, which don't fully represent the tumor microenvironment seen in genetically engineered mouse models (GEMMs). Therefore, we hypothesized that gene expression information would also be useful in selecting appropriate models for study. Indeed, microarray analysis on tumors from the **KRC** PDAC mouse model, which has oncogenic **K**ras and deleted **R**b1 in the pancreas due to **C**re-mediated recombination, revealed enrichment of angiogenesis-related gene ontology terms as well as enrichment of the pro-angiogenic gene signature from the TCGA analysis. Therefore, we propose that KRC mice are an ideal GEMM of PDAC for investigating angiogenic mechanisms and anti-angiogenic therapies directed at PDAC patients with a strong pro-angiogenic gene signature.

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Regulators of the ER-Stress Response Contribute to Survival in Irradiated Glioblastoma Cell Lines

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Therapeutic resistance is a significant barrier to the treatment of glioblastoma multiforme (GBM)—the most common and most aggressive primary brain tumor in adults. Ionizing radiation (IR) is an integral component for the therapy of GBM, however recent studies have shown that IR can induce adaptive responses that may enhance therapeutic resistance and tumor recurrence. We have found that the endoplasmic reticulum stress response (ERSR), a conserved program often deregulated in cancer, can mediate pro-survival signaling in the context of radiation therapy. This signaling may branch directly from the ATF6, IRE1 and PERK pathways—which are the key regulatory arms of the ERSR. In this research, we show for the first time that IR can induce genes downstream of ATF6, IRE1 and PERK in glioblastoma cell lines. Furthermore, we identify the ATF6, IRE1 and PERK pathways as important contributors to cell survival in irradiated glioblastoma cells. We began our study by investigating GRP78 protein and mRNA induction in D54, U87, U118, LN827 and GL261 in irradiated glioblastoma cell lines. Using a fluorescent probe specific for the ER membrane, we observed 50% increased ER staining 48h after IR, which was attenuated by treatment with ROS scavengers. Since GRP78 and ER membrane expansion are known indicators of ER-stress, we hypothesized that ROS generated by IR was disrupting ER-homeostasis. By expressing MERO-GFP, an ER-localized ROS reporter, we found that ER oxidation increased by 37% 72h after 6 Gy. To evaluate the relationship between IR induced changes in ER-homeostasis and downstream ERSR signaling, we performed qRT-PCR to analyze expression of several genes downstream of ATF6, IRE1 and PERK. We found global induction of ER-stress genes in irradiated glioblastoma, and that upregulation of ATF6 target genes after IR could be attenuated with knockdown of ATF6. Similarly, inhibition of PERK revealed a dependence of eIF2a phosphorylation after IR on PERK—suggesting that IR can activate protein signaling downstream of PERK. Using RNA-interference, we found that knockdown of ATF6, IRE1 and ATF4—which is downstream of PERK, significantly reduced proliferation and clonogenic survival. These results indicate that IR induced ER-stress contributes to adaptive survival mechanisms in glioblastoma. Further characterization of the mechanism by which the ERSR can modulate cell survival may reveal novel strategies to improve the outcome of glioblastoma therapies.

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Ceramide Mediated Lethal Mitophagy as a Novel Cell Death Mechanism in FLT3 Targeted Therapy of Acute Myeloid Leukemia

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Mutations in FLT3 receptor tyrosine kinase are common in Acute Myeloid Leukemia (AML) and confer a worse prognosis. FLT3 inhibitors are promising therapeutic agents; however, clinical trials show limited success due to development of resistance. A better understanding of the cell death mechanism in response to FLT3 inhibitors helps in identifying alternative therapeutic strategies. Ceramide, a bioactive sphingolipid, is synthesized de novo by Ceramide Synthases (CerS) and mediates cancer cell death in response to chemotherapeutic agents. This study investigates the biological role of ceramide in AML response to FLT3 targeted therapy. We show that AML patient samples and cell lines expressing FLT3 have suppressed CerS1 expression and lower levels of its product C18-ceramide. Silencing FLT3 expression or its pharmacological inhibition increased CerS1 and C18-ceramide levels while FLT3 overexpression suppressed them. Mechanistically, FLT3 signaling increases the activity of Histone Deacetylase 1 (HDAC1) that prevents the recruitment of Sp1 transcription factor to CerS1 promoter. However, upon FLT3 inhibition, CerS1 promoter becomes associated with acetylated histones recruiting Sp1 and resulting in increased levels of CerS1 and C18-ceramide. The increase in C18-ceramide mediates cell death as silencing CerS1 expression or inhibiting its enzymatic activity protected from FLT3 inhibitors-induced cell death. Mass spectroscopy, confocal, and electron microscopy reveal that the increase in C18-ceramide occurs in mitochondria that form contact sites with autophagosomes. Indeed, FLT3 inhibitors resulted in the formation of LC3B-II containing autophagosomes that co-localize with ceramide and was accompanied by mitochondrial depolarization and decreased ATP generation. LC3B-II has a hydrophobic domain at the amino terminal that binds to ceramide and is required for execution of cell death since disrupting the ceramide-binding domain by overexpressing LC3B-I35A and LC3B-F52A mutants failed to sensitize the cells to cell death mediated by FLT3 inhibition. Interestingly, treatment with C18-pyridinium-ceramide, which accumulates selectively in mitochondria, is able to induce cell death in cells sensitive or resistant to FLT3 inhibitor, through the same mechanism of LC3B-II dependent lethal mitophagy. In summary, our novel data highlights the importance of ceramide metabolism in AML by showing that FLT3 suppresses CerS1 expression in HDAC1 dependent manner, while its inhibition reactivates it leading to the generation of C18-ceramide in mitochondria. C18-ceramide then orchestrates the mechanism of lethal mitophagy by binding to LC3B-II to recruit autophagosomes to mitochondria. The ability of C18-pyridinium-ceramide to target the mitochondria and bypass FLT3 signaling highlights its potential of being established as an alternative therapeutic drug for AML regardless of whether patients are sensitive or resistant to FLT3 targeted therapy.

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Novel Stem Cell Model to Study Cardiac Conduction System Development and Disease

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The cardiac conduction system (CCS) includes a heterogeneous network of cells responsible for initiation and propagation of the heartbeat. The Purkinje fiber network is the most distal portion of the CCS and essential for synchronous ventricular excitation and contraction. A growing body of literature suggests that defects in Purkinje cell (PC) function, either inherited or acquired, can lead to life-threating arrhythmias. However, the scarcity of these cells in the heart, (<1% of ventricular cardiomyocytes), presents challenges for studies of the underlying molecular mechanisms that are responsible for PC specification, differentiation and function in health and disease. MicroRNAs (miRNAs) play essential roles in multiple cell processes including development and disease. We sought to identify miRNAs that might promote PC specification, using an embryonic stem cell model system. We first searched for candidate differentially expressed microRNAs by performing transcriptional profiling of cardiomyocytes from the trabecular (from which PCs arise) and compact myocardium of E10.5, E12.5 and P2 murine hearts, using Affymetrix microarrays. We identified 13 miRNAs that were significantly differentially expressed. To test whether any of these candidates influenced PC differentiation, we transfected each microRNA into genetically engineered murine embryonic stem cells (ESC)-derived cardiac progenitors harboring dual fluorescent reporters: a pan-cardiomyocyte αMHC-mCherry reporter and PC-specific Cntn2-eGFP reporter. The proportion of ESC-derived PCs was quantified by flow cytometry. Transfection with one of the candidates, miR-24, resulted in a significant two-fold increase of dual positive αMHC-mCherry/Cntn2-eGFP expressing putative ESC-derived PCs. Further validation of the role of this miRNA in differentiation and specification of CCS cells during development is currently underway. The understanding of factors involved in the regulation of PC differentiation and function is crucial for the identification of new targeted therapies for diseases of cardiac rhythmicity.

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TRPV1 Residues Vital to Protection Against 4-HNE Modification, Preservation of Channel Activity and Microvascular Function

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We previously demonstrated enhanced 4-hydroxynonenal (4-HNE) post-translational modification (PTM) of TRPV1 decreases TRPV1 functional expression and contributes to microvascular dysfunction in diabetes. Accordingly, we hypothesized that manipulation of residues associated with 4-HNE PTM would preserve TRPV1 function and restore vascular integrity. 4-HNE decreased capsaicin mediated increases in myocardial blood flow and capsaicin-mediated relaxation in isolated coronary microvessels. TRPV1 functional analysis using electrophysiology revealed blunted capsaicin-mediated currents in the presence of 4-HNE which were reversed by the reactive carbonyl species scavenger aminoguanidine (AGD). Using computer modeling we identified three probable residues, previously identified to be important for oxidative modification, as potential sites for 4-HNE modification. The corresponding residues, C616, C621 and C634, were mutated to alanine (individually and in combination) and subsequently we examined the effects of 4-HNE on TRPV1 currents induced by capsaicin via electrophysiology. The mutation of the three pore cysteine (individually or in combination) abrogated the effects of 4-HNE on capsaicin-mediated currents. These data suggest that TRPV1 is targeted by redox-active substances that directly modulate channel activity at numerous sites in diabetes to decrease TRPV1 functional expression and contribute to microvascular dysfunction. The results obtained demonstrate an optimal redox state is critical for a properly functioning TRPV1 channel.

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Decreased SIRT6 in B Cell Malignancies Confers Growth Advantage

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Introduction: B cell malignancies represent a heterogeneous group of diseases. The most common of these is chronic lymphocytic leukemia (CLL), which is characterized by the expansion and accumulation of functionally defective B cells in the blood, bone marrow, lymph nodes and other organs. Sirtuin 6 (SIRT6) localizes to the nucleus, has deacetylase and ADP-ribosyltransferase activities, and has been shown to preferentially hydrolyze long-chain fatty acyl groups. A major target of SIRT6 deacetylation is histone H3K9, and deacetylation of this residue is associated with the function of SIRT6 as a co-repressor of the transcription factor HIF-1α, a critical regulator of nutrient stress responses. Consistent with this notion, SIRT6-deficient cells exhibit increased HIF-1α activity, increased glucose uptake, up-regulation of glycolysis, and diminished mitochondrial metabolism. **Methods**: CLL cells and normal B cells were collected with IRB approval from peripheral blood of CLL patients and healthy donors, respectively. Mantle cell lymphoma (MCL) lymph node tissues were obtained from biopsy. The following B cell malignancy

lines were analyzed: Daudi, Granta-1, JeKo-1, Raji, Ramos, Rec-1, and Z-138. Western blots and immunohistochemistry were used to quantify protein expression, and Q-PCR was used to quantify RNA expression. Spectrophotometric assays were used to quantify glucose uptake and lactate production. CFSE assays were used to assess proliferation**. Results**: SIRT6 protein expression was significantly reduced in CLL and MCL patient samples and a number of B cell malignancy lines compared to primary B cells from healthy donors. Overexpression of SIRT6 was able to partially reverse cancer phenotypes in SIRT6-deficient Raji, Ramos, and Z-138 cell lines. Overexpressing SIRT6 in these lines decreased H3K9Ac levels, cell proliferation, glucose uptake, lactate production, and expression of glycolytic genes. Furthermore, SIRT6-deficient cell lines were more susceptible to the glycolytic inhibitor dichloroactetate (DCA) than their SIRT6-overexpressing counterparts. **Conclusion**: In this first investigation of the role of SIRT6 in B cell malignancies, we have shown that loss of SIRT6 confers a growth advantage to B cell malignancies consistent with the Warburg effect, whereby cancer cells undergo aerobic glycolysis. This suggests that activating the SIRT6 pathway may be of therapeutic interest in treating B cell malignancies.

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Trichostatin A Rescues RPE Function in Diabetic Rats

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Purpose: In diabetic retinopathy a major cause of vision loss is macular edema. The etiology of macular edema has been tied to endothelial dysfunction of the inner blood-retina barrier. However, more recent studies have shown that dysfunction of the retinal pigment epithelium (RPE), which comprises the outer blood-retina barrier, also plays a role in this disease. Changes in protein acetylation have been shown to play a central role in pathophysiological responses to hyperglycemia. In this study, we investigate the effects of the HDAC inhibitor trichostatin A (TSA) on hyperglycemia-induced RPE dysfunction. **Methods**: Brown Norway rats (130-150g) were injected IP with 60 mg/kg of streptozotocin (STZ) dissolved in citrate buffer; controls were injected with buffer alone. Animals were considered diabetic with plasma glucose of 250 mg/dL or greater. Retinal thickness, plasma glucose, and weight were measured. Animals were treated with TSA (2.5 mg/kg BID IP, 10% DMSO) or vehicle control for 4 days before bleb injection. Subretinal blebs (1µL PBS) were created and resorption observed and measured via optical coherence tomography. Rates of fluid resorption were calculated. **Results**: In normoglycemic control rats, the RPE fluid resorption rate was 8.92±1.19 $ul*cm^{-2}*hr^{-1}$(n=4). In diabetic rats, nine weeks after STZ injection, RPE fluid resorption was significantly ($p< 0.05$) reduced to 2.43±0.55 $ul*cm^{-2}*hr^{-1}$ (n=6). In diabetic rats, TSA treatment significantly increased fluid resorption rate to 8.11±1.54 ($ul*cm^{-2}*hr^{-1}$; n=7). In control rats TSA administration had no significant effect on RPE fluid resorption (8.068±1.81 $ul*cm^{-2}*hr^{-1}$; n=4). In diabetic rats, nine weeks after STZ injection, retinal thickness increased by 5.21±1.45% ($p<0.05$; n=4). Diabetic rats treated with TSA exhibited a reduction in retinal thickness (4.38±1.20%; n=9); however, this change did not significantly differ from that observed in untreated diabetic rats. **Conclusions**: Acute TSA treatment normalized RPE fluid resorption in diabetic animals. While a trend towards reducing retinal thickness was measured in this diabetic model, no significant change in thickness was measured with the TSA dosing utilized in these initial studies. These data provide evidence that decreases in protein acetylation plays a role in hyperglycemic-induced RPE dysfunction. However, it is not clear if the use of HDAC inhibitor alone will be efficacious in treating diabetic macular edema.

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Leucine-Rich Repeat Containing Protein 31 (LRRC31), a Novel Regulator of the Esophageal Epithelial Barrier in Eosinophilic Esophagitis

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Eosinophilic esophagitis (EoE), an allergic inflammatory disease of the esophagus, has increased esophageal expression of IL-13. Notably, IL-13-treated primary esophageal epithelial cells exhibit changes in gene expression that markedly overlap with genes differentially expressed in EoE esophageal biopsies. Here, we focus on leucine-rich repeat containing protein 31 (*LRRC31*), a novel gene with increased expression in EoE esophageal biopsies (12-fold, $p<0.05$) and IL-13-treated primary esophageal epithelial cells (26-fold, $p<0.05$). These data led us to hypothesize that IL-13-mediated induction of *LRRC31* is important in EoE pathogenesis. LRRC31 contains 9 leucine-rich repeats, shares highest amino acid identity (28%) with ribonuclease inhibitor 1 (RNH1), has phylogenetic conservation through bony fish, but has no known function. At baseline, *LRRC31* was specifically expressed in airway and colonic mucosal epithelium but not the esophagus. In EoE, esophageal *LRRC31* mRNA expression increased (136-fold, $p<0.05$), normalized in patients responding to therapy, and significantly correlated with *IL13* ($R=0.55$, $p<10^{-4}$) and *CCL26* ($R=0.68$, $p<10^{-4}$) mRNA expression. In IL-13-treated primary esophageal epithelial cells, *LRRC31* was induced in a dose-dependent manner, with peak expression between 24 and 48 hours. However, *LRRC31* mRNA was not induced by IL-13 in submerged cultures of immortalized epithelial cells. We identified IL-13 induction of *LRRC31* mRNA (95-fold, $p<0.05$) in differentiated esophageal epithelial following air liquid interface (ALI) culture. Mechanistically, overexpression of LRRC31 in EPC2 esophageal epithelial cell ALI culture increased barrier integrity as assessed by increased transepithelial electrical resistance (2.63-fold, $p<0.05$) and decreased paracellular flux (3.54-fold, $p<0.05$), suggesting increased barrier integrity. RNA-Seq analysis identified significant changes in 11 genes in LRRC31 overexpression, 3 of which were kallikrein-family proteases (Benjamini-Hochberg FDR $p<0.05$). Kallikrein proteases are known to regulate epidermal stratification, desquamation, and barrier integrity, suggesting a mechanism by which LRRC31 modulates the esophageal epithelial barrier. Thus, we conclude that *LRRC31* is expressed in mucosal epithelium, is induced by IL-13, and regulates esophageal epithelial barrier function by modulating kallikrein protease expression.

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Exploration of Quercetin as a Novel Therapy to Improve Wound Healing

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Background: Poor wound healing and scar formation following surgery or injury can lead to significant morbidity and mortality. Proper wound healing involves integrins and Fas/Fas-ligand(FasL) interactions on fibroblast cells. Quercetin is a flavonoid compound with anti-fibrotic properties that are not well characterized, making it a candidate for scar prevention. However, quercetin's mechanism of action is not well understood. This research seeks to explore the hypothesis that quercetin will decrease wound fibrosis by increasing Fas, FasL and integrins such as αV integrin, β1 integrin and CD47 on fibroblasts, leading to more efficient wound healing. **Methods:** L929 fibroblasts and *ex vivo* skin fibroblast cells isolated from C57BL6/J mice were treated for 24 hours with either quercetin or its vehicle alone. These cells were then analyzed using flow cytometry and microscopy for Fas, FasL, and integrins to examine alterations in their growth potential, likelihood of apoptosis, and adhesiveness. Mice studied were C57BL6/J wild-type(WT) mice and B6.MRL-Fas^{LPR}/J(FL) mice, which have a mutation in their Fas gene and altered skin wound healing. Mice had a 3 millimeter biopsy punch on their back and were treated daily with quercetin or its vehicle alone. At multiple time points wounds were photographed and measured. Trichrome stain was used to characterize the degree of wound fibrosis. **Results:** In L929 cells, cell surface Fas expression increased and levels of FasL decreased in response to quercetin. β1 Integrin, but not αV integrin or CD47, decreased in response to quercetin. These changes indicate altered survival and migratory potential in L929s following quercetin treatment. *Ex* vivo cells demonstrated increased cell surface Fas. *In vivo*, wound healing rates do not differ between the quercetin and control groups in either the WT or FL mice, indicating that quercetin's anti-fibrotic effects do not prevent healing. Trichrome staining of the wounds demonstrates decreased fibrosis, decreased immune infiltrate, and increased cellularity in the wound area in response to quercetin. **Conclusions:** Quercetin could improve wound healing by altering cell makers associated with survival and migration, which might allow cells to enter the wound more efficiently and decrease fibrotic changes in that space. Therefore, it warrants further study for improving wound care. **Conflict of Interest:** M Karen Newell-Rogers receives support from VG Life Sciences.

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Deregulated Expression of Cyclin E Mediates Resistance to Aromatase Inhibitors in Postmenopausal Breast Cancer Patients

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Almost seventy percent of all breast cancer patients have estrogen receptor (ER) positive tumors requiring hormonal therapy. Aromatase inhibitors (AIs) are considered as the first line hormonal therapy for ER+ post-menopausal patients. However, resistance to these drugs remains a major challenge in clinic and the biology of such resistance is not clear. Previous studies have shown that cyclin E pathway, a key regulator in the G1 to S transition of cell cycle, is deregulated in breast cancer. Full-length cyclin E is abnormally cleaved into low molecular weight isoforms (LMW-E) that renders patients to poor survival. Here we hypothesize that cyclin E deregulation can confer resistance to AIs. To address this, we engineered aromatase overexpressing MCF7 cells to overexpress LMW-E under doxycycline inducible promoter. Full-length cyclin E, GFP and empty vector transfected cells were also generated and used as controls. Our results indicated that AIs inhibited proliferation by arresting the cells at G1 phase of the cell cycle. However, LMW-E expression significantly enhanced proliferation of the cells when treated with AIs. In addition, LMW-E bypassed G1 arrest following AI treatment. At the molecular level, AIs decreased CDK2, pCDK2, and Rb levels and attenuated Rb phosphorylation. However, these effects were completely rescued only when LMW-E was expressed. Moreover, using an in vitro kinase assay we indicated that AIs decreased CDK2 kinase activity while LMWE expression reversed this effect by increasing CDK2 enzymatic activity. Knocking down CDK2 or Rb using independent shRNAs disrupted LMW-E mediated resistance further confirming that CDK2-Rb pathway regulates LMW-E response. Taken together, these results suggest that LMW-E inactivates Rb protein as a tumor suppressor and renders the cells to bypass G1 checkpoint following AI treatment. In addition, this study provides early evidence that CDK2 inhibitors could be beneficial in combination with AIs for LMW-E expressing tumors. Currently we are investigating whether LMW-E can bypass the activity of AIs using inducible breast cancer cell line xenograft. We are also examining the correlation between cyclin E status and response to treatment in a cohort of patients who received AIs in the neo-adjuvant setting.

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Primary Care Provider, Peer Advisor, and Patient Reported Barriers to Improvement of Cardiovascular Health for Individuals Living in the Alabama Black Belt

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Despite advances in prevention, management, and treatment, cardiovascular disease (CVD) remains the leading cause of death in the United States, with Alabama among the states having the most alarming statistics. To help combat this disease, the American Heart Association created several recommendations for cardiovascular health improvement, known as "Life's Simple 7", involving keeping blood pressure, cholesterol, blood sugar, and weight in normal ranges, as well as lifestyle modifications including eating a healthy diet, exercising and not smoking. People who reside in Alabama's rural Black Belt region are especially prone to poor health outcomes, including poor measures on Life's Simple 7. Our semi-qualitative research studied the barriers to achieving Life's Simple 7 in the Alabama Black Belt from the perspective of 3 stakeholder groups (primary care providers, peer advisors, and patients) using the nominal group technique (NGT). The NGT is a form of information gathering in which a facilitator, after posing a question, solicits responses from all participants, thereby generating a visible list for further evaluation. The result of each nominal group is a prioritized list of barriers, from the perspective of that group's participants. The top priority barriers from each group were standardized across all groups and categorized using the Socio-Ecological Framework, which conceptualizes barriers at the individual, interpersonal, organizational, community, and societal levels. Results portrayed a high level of agreement between the nominal groups of the peer advisors and patients. Lack of concordance between the two physician nominal groups suggests our inability to reach saturation. Peer advisors focused on barriers that they could specifically help patients with, namely those at the interpersonal and community levels, whereas patients tended to focus on their own personal barriers. Physicians portrayed a more holistic understanding of barriers, citing individual, interpersonal, organizational, and community level barriers. It is imperative to engage and understand the perspectives of stakeholders prior to intervention development; this study revealed that the three stakeholders emphasized barriers at different socio-ecological levels. To maximize engagement, these results can be used to design an intervention that addresses the expressed concerns of each stakeholder.

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Gain of ATXN1 Function Contributes to Dysregulation of Postnatal Cerebellar Stem Cells in Spinocerebellar Ataxia-1

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Spinocerebellar Ataxia-1 (SCA1) is an autosomal-dominant neurodegenerative disorder caused by polyglutamine expansion in the protein ataxin1 (ATXN1). Characterized by mid-life cerebellar degeneration with ataxia as the presenting symptom, SCA1 is relentless, and patients eventually succumb from the disease. Work from mouse models suggest that even though the symptoms are relatively delayed, pathogenic processes begin surprisingly early—indeed as early as the first 1-2 weeks of postnatal life. It is tempting to speculate that pathogenic changes in neurons interfere with other contemporaneous developmental processes. In this context, we have been intrigued by the recent findings that there exists a pool of stem cells in the postnatal cerebellum that contribute to cerebellar development. It is important to note that these stem cells are distinct from the relatively well characterized stem cells of the olfactory and ventricular zones elsewhere in the brain. We hypothesize that mutant ATXN1 interferes with this stem cell pool either through their toxic properties on stem cells per se, or by a non-autonomous effects from affected neurons through factors such as VEGF known to be down regulated in SCA1, so as to compromise cerebellar development and thus contribute to disease later in life. To test this hypothesis, we have begun to characterize the postnatal cerebellar stem cells from SCA1 knock in (154Q), BO5 (84Q, Purkinje cell specific expression) and Atxn1 knockout mice. Surprisingly we found that the stem cells from SCA1 (154Q) show reduced proliferation and premature differentiation capacity, where stem cells from BO5 (84Q) and Atxn1 knockout mice acts as wild type cells. We also found the molecular targets behind the deregulation of mutant SCA1 stem cells that might be playing important role in neuronal differentiation. From above results it is clear that the dysregulation of SCA1 (154Q) stem cells took place due to gain of mutant ataxin-1 function and it is cell autonomous. As a corollary to this hypothesis, we also propose that harnessing this stem cell population either during development or later in life could benefit the symptoms of this disease and by extension potentially other cerebellar disorders.

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Blocking Human Rhabdomyosarcoma Growth in Xenograft Mice by Targeting Gliomas-Associated Oncogene Transcription Factors

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Rhabdomyosarcoma (RMS) is the most common form of soft tissue sarcoma in children. Current theraputic measures have failed to improve the cure rates for patients with recurrent and metastatic disease. Previous attempts of inhibiting the sonic hedgehog pathway using upstream effector molecules have been relatively unsuccessful. These molecules targeted the smoothened (Smo) protein. Resistance to these molecules has been attributed to activation of downstream molecules such as gliomas-associated oncogene transcription factor-1 (Gli-1), Gli2&3. In this study we have investigated the effect of Gant-61, a Gli-1&2 inhibitor on established Human RMS cell lines RD (embryonal RMS) and RH30 (alveolar RMS) both *in vivo* and *in vitro*. Our data showed , *in vivo,* compared to vehicle-treated control group, more than 53% (RD cells) and 47% (RH30 cells) inhibition in xenograft tumor growth was observed in mice receiving intra peritoneal injection of Gant-61 (50mg/kg) three times a week. Gant-61-treated group showed significant reduction in the expression of biomarkers related to cell proliferation i.e. PCNA, Cyclins as well as decrease in tumor invasiveness markers such as fibronectin, twist and snail. Gant-61-treated tumors also found to induce more apoptosed cells as measured by TUNEL assay. Our *in vitro* data in both RMS cell lines support the above findings as treatment with Gant-61 (5-25 micromolar) showed concentration-dependent reduction in inhibition of Gli-1, 2 and 3, transcriptional factors. Interestingly, Gant-61 exposed RMS cell lines also sohw diminution in expression level of the phosphorylation of Akt (upstream) and 4EBP1 and p70S6K (downstream) markers of mTOR signaling pathway. Targeting Gliomas-associated oncogene transcription factors represent a novel target in both alveolar and embryonal rhabdomyosarcoma and their blockade diminish the growth of these tumors in xenograft model. Gant-61 represent a novel molecule with possible indications to be tested in combination with other known chemotheraputic agents to improve cure and long term survival.

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Entry and Immunomodulation: Exploring the Dual Roles of the HVEM Receptor in Ocular Herpes Simplex Virus Type 1 Infections

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Herpes simplex virus type 1 (HSV-1) can infect the ocular tissues, producing significant immune-mediated tissue damage. Recurrent bouts of this immuno-inflammatory syndrome, herpes stromal keratitis (HSK), can eventually result in blindness. Herpes virus entry mediator (HVEM) is a TNF-receptor superfamily member and functions as an HSV entry receptor through its interactions with the viral envelope glycoprotein gD. Nectin-1, another herpesvirus entry receptor, also binds gD at a site independent from that of HVEM. HSV-1 infection of the eye uniquely depends on HVEM, as HSV-1 infection is attenuated in HVEM knockout (KO) mice after corneal inoculation. Because HVEM bound by its natural ligands has a variety of immunomodulatory effects, both pro- and anti-inflammatory, I hypothesized that non-entry functions of HVEM drive HSV-1 pathogenesis in the eye. Deletion of amino acids 7-15 of HSV-1/17 gD produced a virus capable of utilizing nectin-1 but not HVEM for entry. C57Bl/6 mice infected with HSV-1/17gDΔ7-15 exhibited comparable clinical symptoms, neurologic morbidity, and viral loads in tear film and tissues as mice infected with a repaired control virus with intact HVEM entry (HSV-1/17WT-FRT). Analysis of corneal cytokines and chemokines 5 days post infection (dpi) revealed no differences between corneas from WT mice infected with gDΔ7-15 or WT-FRT control viruses. In contrast, corneas from HVEM KO mice infected with the parental HSV-1/17 virus expressed several factors, including IL-6, MIP-1α, and RANTES, at 5- to 75-fold lower levels than WT. These data indicate that HVEM expression changes the immune response to HSV-1 in the eye, and that the contribution this receptor makes pathogenesis is likely gD- and entry-independent. Chimeric mice with HVEM expression restricted to or ablated from bone marrow derived lineages (ie immune cells) were also infected via corneal scarification. As expected, control HVEM KO mice that received HVEM KO marrow (hvem ko/HVEM KO) had lower lesion scores, neurologic scores, and mortality compared to wt/WT controls. HVEM KO mice that received WT marrow (wt/HVEM KO) were protected from infection and were indistinguishable from hvem ko/HVEM KO controls. Together, these data suggest that HVEM on radiation-resistant cell types has important immunomodulatory role(s) that contribute to ocular HSV-1 pathogenesis. Further studies are underway to identify the specific HVEM-expressing cell type(s) of importance and explore how HVEM expression on these cell type(s) influences the immune response to HSV-1 infection of the eye. Because antivirals like acyclovir control HSV replication but do not address the pathologic inflammatory response of the host, HVEM may be an appealing target for novel HSK therapeutics.

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Downregulation of Menin by miR-24-1 Plays a Key Role in the Regulation of Cholangiocarcinoma Proliferation

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Cholangiocarcinoma (CCA) is a devastating cancer originating from biliary epithelium with poor prognosis. Menin is a nuclear protein that is encoded by MEN1 (multiple endocrine neoplasia type 1) tumor suppressor gene. Since CCA displays a neuroendocrine phenotype and secretes and responds to a number of hormones and neuropeptides, we tested the hypothesis that menin regulates CCA proliferation. **Methods:** Menin expression was evaluated by *q*PCR, Western blot and immunofluorescence in human cholangiocytes, both malignant and nonmalignant, and by immunohistochemistry in human CCA tissue arrays. The effects of menin expression on proliferation and VEGFA expression levels were evaluated in cells transfected with menin siRNA (24 and 48 hr) by MTS assay and qPCR, respectively. We examined miR-24-1 expression levels with real-time PCR as well as menin expression and cell proliferation after miR-24-1 knockdown with hairpin inhibitors with real-time PCR and MTS, respectively. **Results:** Menin expression levels were significantly reduced in all the malignant cell lines compared to healthy cells as well as human CCA tissue compared to nonmalignant controls. Knockdown of menin expression with siRNA significantly increased the proliferation and VEGFA. The expression levels of miR-24-1 were increased in CCA cell compared to healthy cells. Inhibition of miR-24-1 with specific hairpin constructs resulted in increased menin expression levels and decreased proliferation. **Summary/Conclusion:** We have shown that the expression of the tumor suppressor menin may be important in the regulation of non-malignant and CCA proliferation. Local modulation of menin within cholangiocarcinomas may be an additional therapeutic tool for managing cholangiocarcinoma progression.

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Bile Acids Promote Proliferation Through the Scaffolding Protein IQGAP1

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Liver cancer is the second leading cause of cancer-related death worldwide with hepatocellular carcinoma (HCC) being the major subtype. The primary risk factors for HCC such as cirrhosis and hepatitis B and C infections are associated with elevated serum bile acids, which are produced by the liver and are known for their role in lipid absorption. Recently, bile acids' ability to function as a signaling molecule via receptors as well as modulating kinase signaling has also been elucidated. Previously, we found that Fxr$^{-/-}$Shp$^{-/-}$ mice, which have lost their feedback regulation of bile acid synthesis, accumulated excessive bile acids and spontaneously developed HCC. Unexpectedly, these mice, as well as cholic acid-fed wild-type mice, displayed dramatic increase in hepatic expression of IQGAP1, a scaffold protein that has been shown to promote proliferation in other tissues such as breast epithelium. Moreover, cholic acid-fed *Iqgap1*$^{-/-}$ mice displayed fewer proliferating cells compared to wild-type mice as indicated by Ki-67 stain indicating a crucial role for IQGAP1 in promoting bile acid-dependent proliferative response. Based on these results, **we hypothesize that bile acids employ IQGAP1-mediated mitogenic signaling to promote liver carcinogenesis.** To address this hypothesis, we examined pro- and anti-proliferative signals in response to cholic acid. EGFR, ERK, and beta-catenin mitogenic signals were activated with cholic acid treatment as expected but in a IQGAP1 independent fashion. On the other hand, we found that the cholic acid-mediated reduction in anti-proliferative hepatic signals including integrin-linked kinases, E-cadherin, and phospho-SMAD3 were lost in *Iqgap1*$^{-/-}$ mice. Furthermore, we have validated bile acid-induced upregulation of IQGAP1 in vitro. We summarize that IQGAP1 functions downstream of bile acid signaling and plays a key role in regulating the proliferative response. Therefore, understanding this new bile acid-IQGAP1 axis may provide novel ways to control undesired proliferation.

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Myristoylated Alanine Rich C-Kinase Substrate (MARCKS) has a Critical Role in the Growth and Proliferation of Glioblastoma Multiforme

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Background: Glioblastoma multiforme (GBM) is the most common and deadly form of Glioma, with a median survival of 14 months despite maximal surgical resection, chemotherapy and radiation. A loss of heterozygosity (LOH) of chromosome 10q has been found in 90% of GBM to date and a mutation in the tumor suppressor Phosphatase and Tensin Homolog (PTEN) is combined with this LOH in 60% of these cases [2]. PTEN has its tumor suppressor function by antagonizing PI3K/Akt signaling which begins when PI3K phosphorylates Phosphatidylinositol (4,5)-bisphosphate (PIP2) into Phosphatidylinositol 3-kinase (PI3K) allowing for AKT activation. PIP3 recruits AKT to the plasma membrane where it phosphorylated and leads to changes in migration, invasion, angiogenesis, survival and proliferation. PTEN is responsible for dephosphorylating PIP3 back into PIP2; whereas Myristoylated Alanine Rich C-Kinase Substrate (MARCKS) electrostatically sequesters PIP2. It has been shown that activating mutations of PI3K, deactivating mutations of PTEN and reduced levels of MARCKS all correlate with worsened GBM patient survival. MARCKS expression has been shown to be strongly correlated with increased patient survival. **Hypothesis:** MARCKS is a key regulator of GBM growth and sensitivity, therefore, increasing levels of phosphorylated MARCKS in combination with DNA damaging Temozolomide (TMZ) and/or radiation therapy (RT) in GBM cells, will further suppress cell growth. **Methods:** MARCKS mutants were created using a GBM cell lines with low native MARCKS expression (U87) using a tetracycline inducible lentiviral vector. We created cell lines that will express

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MARCKS with a wild type (WT) effector domain(ED), a pseudo-phosphorylated (PP) ED, and non-phosphorylatable (NP) ED and one without an ED (deltaED). Cells were plated and subsequently treated with doxycycline, TMZ and RT. Cell viability after one week was assessed by an ATP-light, Clonogenic, and Real-time cell-impedance sensing (RT-CES) assay. **Results:** Increasing MARCKS expression in combination with TMZ therapy resulted in significant tumor growth suppression. **Conclusions:** Further investigation into the function and regulation of MARCKS activity can aid the development of potential GBM combination therapies.

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Endotoxin Treatment Enhances the Metabolic Response to Infection

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The Sherwood lab recently discovered that animals treated with lipopolysaccharide (LPS, AKA endotoxin) or its less toxic derivative monophosphoryl lipid A (MPLA) are non-specifically protected from subsequent infection. Due to it's safety profile, MPLA is currently being investigated as a pharmaceutical intervention aimed at decreasing infections in high-risk patients. In spite of this protection, treatment with LPS or MPLA is well known to suppress the cytokine response to future infection, and the presence of LPS on bacteria was once thought to predispose infected patients to secondary infections via immunosuppression. Why LPS or MPLA treatment paradoxically protects against infection is unknown, but macrophages appear critical to coordinating this effect. Here, we exposed bone marrow derived macrophages to MPLA or LPS and, after a significant washout period, measured their response to LPS. We found that LPS and MPLA both primed the macrophages to respond metabolically to LPS as measured by lactate production and glucose consumption. Further, while cytokine secretion and NFkB signaling were expectedly diminished, metabolism-related phosphoinositide 3-kinase (PI3K) signaling remained intact. This study challenges the notion that endotoxin tolerant animals are truly 'tolerant' to all effects of endotoxin. Further, metabolic priming may be a primary mechanism by which LPS and MPLA protect against future infection.

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High-Dimensional Mass Cytometry Analysis of Acute Myeloid Leukemia Therapy Response

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Acute myeloid leukemia (AML) is increasingly understood as a disease where subpopulations, and perhaps individual cells, respond differentially to therapy. Given this plasticity and heterogeneity, a high content single cell approach was designed to pinpoint populations of AML cells that are rare pre-treatment but emerge and dominate following therapy resistance. We characterized 1) abundance of rare AML cell subsets, 2) kinetics of early therapy response, and 3) emergence of novel leukemic populations using 27 diagnostic and differentiation markers measured by mass cytometry (CyTOF). AML patients undergoing induction chemotherapy were identified and consented for sample collection. Samples of peripheral blood and bone marrow were collected throughout induction therapy and cryopreserved after mononuclear cell separation from 5 AML patients before collection on CyTOF and analysis of cell phenotypes by viSNE. Leukemic blasts were $CD45^{lo}$ and comprised phenotypically distinct groups of cells. viSNE analysis of the 27-marker panel grouped cells into 11 major populations: $CD34^{+}$ hematopoietic stem/progenitor cells (HSCs), 5 differentiated non-malignant populations (myeloid, $CD4^{+}$ T, $CD8^{+}$ T, B, dendritic and NK cells), and 2 distinct populations of leukemia cells. Distance of individual cell populations from the HSC population was measured and was lower for AML than for mature cell populations. Clinical remission was consistent with mass cytometry data (defined by <5% blasts). In a case of primary refractory disease, seven persistent cell populations became dominant post-therapy. These same persistent populations were rare pre-treatment (<10% of AML blasts). Persisting AML blasts became more phenotypically distinct from stem and progenitor cells due to expression of novel marker patterns that differed from pre-treatment AML cells and from all cell types observed in healthy bone marrow. These results demonstrate the ability of high content mass cytometry to reveal, characterize, and compare rare and highly plastic cell populations over time in primary human tissue samples. Critically, use of viSNE and 27 markers meant that cells that dramatically changed expression of a few markers could still be matched to phenotypically similar cells based on similar expression of the other 20+ markers. This approach offers greatly expanded longitudinal characterization of AML while effectively capturing differentiation and cell subset dynamics.

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Preoperative Multiparametric Prostate MRI Alters Nerve Sparing Decision Making While Optimizing Oncologic Outcomes in High Risk Patients

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Introduction and Objective: Preoperative multiparametric magnetic resonance imaging (mpMRI) provides critical data including local staging, delineation of anatomic information, and identification of high risk features such as extracapsular extension. This aids in treatment, counseling, and operative planning. Nerve sparing in robotic assisted radical prostatectomy (RARP) leads to greater postoperative potency and decreased incontinence, but with risk of positive surgical margins. There is little data showing definite mpMRI utility in nerve sparing and its effects on surgical decisions. The purpose of this study is to analyze the utility of mpMRI in surgical decision making, namely excision of nerve bundles as well as its impact on surgical margins. **Methods:** This retrospective study analyzes preoperative mpMRI use in RARP patients at Loyola University Medical Center (n=24) and a simultaneous cohort of RARP patients who did not have mpMRI (n=72). We collected demographic and clinicopathological data, mpMRI findings, postoperative pathology and noted surgical decisions. Patients were stratified by their National Comprehensive Cancer Network risk and the impact of mpMRI on the decision to spare or excise nerve bundles was analyzed. **Results:** A larger percentage of the mpMRI group was high risk (38% compared to 21% of non MRI) and intermediate risk (46% compared to 42%). Conversely, a larger proportion of the non MRI group was low risk (38% compared to 17% MRI). The mpMRI group had higher nerve sparing than the non MRI group in high risk (66% compared to 47%), lower in intermediate risk (66% compared to 100%), and higher in low risk (100% compared to 93%). The mpMRI group had higher bilateral nerve sparing than the non MRI group (33% compared to 7%). The impact of mpMRI on nerve sparing was assessed; 13% of cases were treated more conservatively (i.e. no nerve sparing) and 17% of cases were treated more aggressively. Negative margin rates were better in the mpMRI for pT2 patients (83% compared to 81% for) and for pT3 patients (67% compared to 54%). Lastly, the sensitivity and specificity of mpMRI for extracapsular extension was 54% and 82% respectively. **Conclusions:** mpMRI aids surgical decision making in high risk RARP, allowing for higher nerve sparing rates and appropriately alters decision making in terms of neurovascular bundle preservation and excision without compromising oncological outcomes. Further study is warranted to improve current approaches to RARP, accuracy of mpMRI, and the decision making in high risk patients.

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The Neuroprotective Potential of Subthalamic Nucleus Deep Brain Stimulation in an α-Synuclein Overexpression Rat Model of Parkinson's Disease

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Deep brain stimulation (DBS) is the most common neurosurgical treatment for the alleviation of Parkinson's disease (PD) motor symptoms. Beyond symptomatic efficacy, our laboratory and others have demonstrated that high-frequency DBS of the subthalamic nucleus (STN) provides neuroprotection for dopaminergic neurons of the substantia nigra (SN) in the 6-hydroxydopamine (6-OHDA) rat model of PD. However, the evaluation of neuroprotective strategies in neurotoxin models of PD is limited by the fact that these models primarily assess the ability of neuroprotectants to attenuate oxidative stress, only one of many factors implicated in PD pathophysiology. A large body of evidence points to alpha-synuclein (α-syn) involvement in PD, including the fact that point mutations and multiplications of the *SNCA* gene have been linked to onset of familial forms of PD. In the present study, we investigated whether STN DBS can prevent nigrostriatal degeneration produced by α-syn-mediated neurotoxicity. α-syn overexpression targeted to the nigrostriatal system via direct, intranigral injections of α-syn viral vectors results in a neuropathological and behavioral phenotype that recapitulates key features of PD. Young-adult, male rats received two, 2.0 µl, unilateral, intranigral injections of recombinant adeno-associated virus pseudotype 2/5 (rAAV2/5) expressing human wildtype α-syn (rAAV2/5-α-syn, 1.2×10^{13} genome copies per ml). In our laboratory, these rAAV2/5-α-syn injection parameters result in ≈40% and ≈60% SN loss within one and two months, respectively. Rats were implanted ipsilaterally with a DBS electrode in the STN 18 days following vector injection. Rats were assigned to either Active stimulation or no stimulation (Inactive) groups. Active rats received continuous STN stimulation for four weeks (130 Hz, 60 µs, amperage adjusted below the level of dyskinesias). Inactive rats received no stimulation during the same four-week interval and served as a critical control for the effects of electrode implantation. Forelimb use asymmetry will be assessed at the conclusion of the four-week stimulation interval both during stimulation and 24 hours after its cessation. Postmortem outcome measures will include assessment of nigrostriatal α-syn transduction and stereological quantification of tyrosine hydroxylase immunoreactive (THir) SN neurons. Electrode placement will be verified using Kluver-Barrera histochemistry. These pending results will determine whether STN DBS can ameliorate functional deficits induced by α-syn overexpression or provide neuroprotection from α-syn-mediated nigrostriatal degeneration. *Supported by Spectrum Health and the Morris K. Udall Center of Excellence for Parkinson's Disease Research at Michigan State University.*

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The Impact of Phosphorylation and Oligomerization on Alpha-Synuclein Induced Vesicle Rupture and its Implications for Parkinson's Disease Pathogenesis

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Numerous recent studies have increasingly implicated smaller aggregates or oligomers of alpha-synuclein (a-syn) as the pathological cause of Parkinson's disease (PD). Furthermore, it is now becoming appreciated that aggregated a-syn can spread from cell to cell in a prion-like fashion, propagating its misfolded conformation and higher order aggregation state from affected neurons to neighboring cells in much the same way as a spreading bacterial or viral infection. Understanding the cellular and molecular mechanisms responsible for this transfer between cells is critical to developing treatment approaches designed to arrest or prevent disease progression. Our lab has previously demonstrated that rupture of lysosomal membranes following endocytosis is a mechanism by which a-syn is able to spread from cell to cell in an "infectious" way, inducing cathepsin-mediated oxidative stress and inflammasome activation. In order to further investigate the ability of a-syn to rupture vesicular membranes and access the cytosol after endocytosis, and to test the hypothesis that familial missense mutations or phosphorylation can differentially affect the efficacy of a-syn entry in this manner, we generated aggregates of purified WT, p-S129 WT, E46K, A53T, A30P, p-S129 A30P, and G51D a-syn and measured the ability of each of these species to induce vesicle rupture using the galectin-3 redistribution assay as previously described. Complementary biochemical characterization of each a-syn species after aggregation utilizing SDS- and native-PAGE, EM, and K114 staining has aided in understanding the size-distribution of various a-syn preparations, which has facilitated the correlation of changes in aggregation state with effectiveness of vesicle rupture for each a-syn species. Results thus far suggest that smaller aggregates of each type of a-syn are able to significantly induce vesicle rupture, and differences in vesicle-rupturing ability between the various types of a-syn are being elucidated. Additionally, different phenotypes of vesicles exhibiting rupture by a-syn have been observed, and subsequent investigation of these various populations of vesicles utilizing immunohistochemical staining of vesicle antigens EEA1, LAMP2, and LC3 for early endosomes, lysosomes, and autophagosomes has provided further mechanistic insight into the movement of a-syn within and between cells, and the detrimental consequences of this spread for affected neurons. Increasing knowledge regarding the ability of misfolded a-syn to induce pathology and the mechanisms underlying its spread from one neuron to the next will ultimately aid in the development of disease-modifying therapeutic strategies to prevent or reverse the progression of this devastating neurodegenerative disease.

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The MitoInteractome: Characterization of Disease-Associated Orphan Mitochondrial Proteins

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Mitochondria are organelles whose function is central to human health, and whose dysfunction has been linked to more than 150 diseases. Despite several core functions of mitochondria being established five decades ago, many questions still remain. Recent technological advancements have quadrupled the number of known mitochondrial proteins, hundreds of which have no established biochemical function. At least 40 of these uncharacterized, "orphan" proteins are associated with human diseases, including several which are essential for the biosynthesis of coenzyme Q (CoQ). Our goal is to establish functions for these disease-relevant orphan mitochondrial proteins through the use of large-scale proteomics and focused biochemistry. We first expressed epitope-tagged variants of nearly 100 orphan protein "baits" in each of two cell lines. The bait proteins were purified using an anti-FLAG tag resin, and any co-purifying proteins were identified and quantified using state-of-the art label-free LC-MS/MS proteomics. In this way we can compare the abundance of each "prey" protein across every bait experiment and identify preys that are highly-enriched with each bait. To assess the potential regulatory and dynamic nature of these interactions, we have measured their abundance in cells grown in either glucose and galactose as the carbohydrate carbon source. This is a known, reproducible, metabolic switch between a glycolytic (low-mitochondrial activity) and oxidative (high-activity) state. The initial results from our pipeline of expression, purification, quantification, and analysis have led to the validation of putative complexes as well as the identification of novel, essential interactions. In the former category, we have revealed a densely-connected CoQ biosynthetic protein complex in humans. Many of the interactions among the COQ proteins are modulated by carbon source switching. Additionally, we have implicated several orphan proteins as regulators of key processes such as electron transfer and protein quality control, pathways that are defective in glutaric aciduria and spastic paraplegia, respectively. These results hold promise for the identification of new pharmacologic therapies for diseases currently lacking treatment, while simultaneously advancing the forefront of mitochondrial biochemistry.

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Targeting Hidden Binding Sites in a Cancer Relevant Kinase

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Protein tyrosine kinases are centrally involved in many vital cellular signaling processes. Due to their prevalence, strict regulation of kinase activity is necessary to control essential processes such as the cell cycle, proliferation, differentiation, motility, and cell death or survival. Unregulated kinase activity underlies many diseases including cancer and diabetes. Since the aberrant signaling pathway is known in these diseases, it is possible to target it with small molecule kinase inhibitors. These specific kinase inhibitors have been shown to be powerful drugs. Up to this point, all small molecule kinase inhibitors in clinical use target the ATP-binding pocket. The high sequence and structural conservation of this pocket creates a challenge to developing specific inhibitors. Specificity and resistance are two hurdles that face ATP competitive inhibitors as effective drugs. A method of drug design that could overcome both of these issues is allosteric inhibition. Allosteric sites on kinases are often very specific to a particular kinase and may be targeted with high affinity and high specificity ligands. Furthermore, allosteric regulatory sites can have either an activating or inhibiting function. Unfortunately, it is challenging to identify allosteric sites. Recent computational studies demonstrated that it is possible to predict binding sites for a well-characterized Src-family kinase inhibitor (PP1) in addition to the experimentally determined binding site. Consequently, the simulations found that the ligand resided for surprisingly long times at a previously undescribed binding patch on the surface of Src kinase. The goal of this work was to find ligands that preferentially bind and stabilize this patch. In this study, we performed a comprehensive virtual screen of 232,000 compounds using DOCK6.6. From the results of the virtual screen, we biochemically tested 100 compounds. From these 100 compounds we identified and characterized several inhibitors. These inhibitors are specific to Src kinase over highly related kinases. They also are non-competitive with ATP or substrate. This study provides a proof of principle that it is possible to predict allosteric binding sites and their ligands in the kinase domain, providing a route towards future development of novel cancer therapeutics.

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The Cardiac and Pulmonary Vein Protein Alpha-T-Catenin Contributes to the Pathogenesis of Occupational Asthma

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Rationale: 10-25% of adult asthma is occupation-induced, but its pathogenesis is not well described. Recently, a genome-wide association study identified single nucleotide polymorphisms (SNPs) in the cardiac adhesion protein αT-catenin (αT-cat) that correlated with the incidence and severity of toluene diisocyanate (TDI) occupational asthma. αT-cat is also implicated in regulating overall lung function, with analysis of a pediatric cohort identifying two additional SNPs in aT-cat correlated with changes to forced vital capacity. aT-cat is an essential subunit of the cadherin adhesion complex, mediating strong intercellular adhesion in cardiomyocytes. However, how the dysfunction of this cardiac protein may contribute to asthma or lung mechanics is unknown. **Methods:** αT-cat knockout (KO) mice, which develop an age-related cardiomyopathy, and wild-type (WT) littermates were used to identify the primary αT-cat-expressing cell type in the lung. To examine changes in lung mechanics due to αT-cat, we ventilated WT and KO mice using the Flexivent system. To test αT-cat's role in asthma, we used a murine model of TDI-asthma with an intranasal sensitization and nebulized challenge. **Results:** We found that αT-cat is absent in the airways of the lung, but present within the cardiac sheath surrounding the pulmonary veins. By ventilation, KO mice had a significantly altered pressure-volume curve. This was quantified by calculating the area within the curve, which was significantly higher in KO mice. In the occupational-asthma model, TDI-exposed αT-cat KO mice show increased airway hyperresponsiveness when compared to WT mice. Interestingly, bronchoalveolar lavage revealed only a mild macrophage-dominant inflammation that was not significantly different between WT and KO mice. Further analysis of histological sections by H&E revealed that although PV are adjacent to the large airways, no differences in airway inflammation are observed between WT and KO mice. **Conclusions:** We show that the cardiac protein αT-cat contributes to the development of TDI-asthma. Since we observed no inflammatory differences between WT and KO mice, our data suggest a novel mechanism for the pathogenesis of occupational asthma. Because the pulmonary veins can be found near the large airways, we hypothesize that cardiomyocyte adhesion dysfunction in the heart and pulmonary veins may lead to interstitial airway edema. We also found that αT-cat significantly affects lung hysteresis at baseline, which indicates that the heart and PV may affect normal lung function. Overall, our study indicates that it may be beneficial to screen TDI-exposed workers for cardiac dysfunction and αT-cat SNPs to prevent the development of occupational asthma.

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Ets Homologous Factor Regulates Pathways Controlling Response to Injury in Airway Epithelial Cells

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The airway epithelium plays a critical role in lung function by forming a barrier between the external and internal environment, releasing inflammatory mediators in response to injury or pathogens, and clearing foreign particles through the mucociliary escalator. Disruption of this tissue is central to many diseases of the respiratory system, including cystic fibrosis (CF), asthma, and chronic obstructive pulmonary disease. The response of the lung epithelium to the environment is mediated by a network of transcription factors. Ets homologous factor (EHF) is an Ets family transcription factor expressed in the epithelial cells lining the respiratory tract. Its expression is increased in the bronchial epithelium in response to the presence of inflammatory mediators. *EHF* maps to the 5′ end of an intergenic region that contains single nucleotide polymorphisms identified in a genome-wide association study to associate with lung disease severity in CF patients, suggesting it may play a role in modification of the lung phenotype in this population. EHF can act as a transcriptional activator or a repressor, however its targets in lung epithelial cells are largely uncharacterized. We utilized high-throughput methods to identify EHF targets genome-wide in the Calu-3 lung adenocarcinoma cell line. Chromatin immunoprecipitation followed by sequencing (ChIP-seq), showed that the majority of EHF binding sites are intronic or intergenic and found in putative *cis*-regulatory elements, marked by specific histone modifications and increased DNase hypersensitivity. EHF occupies many genomic sites that are near genes involved in epithelial adhesion. RNA-seq after EHF modulation showed significant alterations in the expression of genes involved in response to wounding, epithelial development and differentiation, and locomotory behavior. These changes in gene expression translated to alterations in epithelial cell phenotype. EHF depletion slowed wound closure and increased transepithelial resistance, a measure of epithelial barrier maintenance. These data suggest that EHF regulates genes in pathways critical for the lung epithelial response to injury, including wound closure, release of inflammatory mediators, and cell adhesion.

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Adjuvant Radiotherapy for Pathologically Advanced Prostate Cancer Improves Biochemical Recurrence Free Survival Compared to Salvage Radiotherapy

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Introduction And Objectives: The proper timing of radiation therapy following radical prostatectomy (RP) for adverse pathologic features and an undetectable PSA remains in question. We evaluate the long-term outcomes of patients receiving adjuvant radiotherapy (ART) and salvage radiotherapy (SRT). **Methods:** A retrospective review of patients receiving RT following prostatectomy at a single institution between 1992 and 2013 was performed. All patients were categorized by adverse pathologic features (Gleason ≥ 8, pT3-4, seminal vesicle invasion, extracapsular extension, and/or positive surgical margins). All patients had an undetectable PSA immediately after RP. ART patients received therapy with undetectable PSA, while SRT patients received therapy post-RP based on biochemical recurrence (BCR). Post-RT BCR, overall survival (OS), bone metastases (BMet), and hormonal therapy (HT) were assessed. Kaplan-Meier curves and Cox stepwise multivariate regression were performed. **Results:** Post-RP patients (n=134) received either ART (n=47) or SRT (n=87). Median age at RP was 60 years, median age at RT was 63 years, and median follow-up was 53 months. Five-year BCR-free survival was 78% for ART vs 50% SRT (HR 3.4, p=0.002). Patients with RT administered following a detectable PSA (SRT) had an increased risk of BCR compared to undetectable: PSA >0.0-0.2: HR 4.1, p=0.005, and PSA >0.2-1.0: HR 4.4, p=0.003. Patients with a pre-RT PSA >1.0 were associated with the highest risk of BCR (HR 52, p<0.001), BMet (HR 40, p=0.019), and HT (HR 68, p<0.001). In the entire group, pathologic Gleason score 8-10 was associated with BCR (HR 3.1, p=0.022) and the need for HT (HR 4.9, p=0.012). There was no demonstrable difference in OS, BMet or HT between ART and SRT. **Conclusions:** ART improves BCR-free survival compared to SRT in patients with adverse pathologic features and an undetectable post-RP PSA. When pre-RT PSA becomes detectable, there is increased risk of BCR. Once pre-RT PSA >1.0ng/mL, there is an increased risk of BCR, BMet, and HT.

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Modeling Stromal-Epithelial Interactions to Assess Homeostatic and Carcinogenic Influences of 17β-Estradiol on the Prostate Stem Cell Niche

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Background: Estrogen is implicated in normal prostate development and prostate carcinogenesis. Prostate stem and progenitor cells express robust levels of estrogen receptors α and β and are direct targets of 17β -estradiol (E2), which stimulates their symmetric self-renewal. Since prostate stromal cells (PrSCs) also express ERα and are known mediators of hormonal action in the prostate, we sought to determine if estrogen acts indirectly through PrSCs to modulate the normal epithelial stem cell niche. **Methods**: Using a 3-D prostasphere (PS) culture system, we enriched stem and progenitor cells from primary human prostate epithelial cells obtained from disease-free adult donors. We modeled stromal-epithelial interactions by culturing PS with either 1) vehicle- or E2-stimulated stromal cell conditioned media (SCCM) or 2) PrSCs in a direct co-culture system with vehicle or E2. Expression of epithelial stemness and differentiation genes was analyzed by reverse-transcription qPCR. Additionally, a novel BrdU label-retention assay enabled measurement of stromal and estrogenic influences on stem cell symmetric self-renewal. Finally, we compared basal gene expression between PrSCs and cancer associated fibroblasts (CAFs) to guide future studies on how E2 might impact the cancer stem cell niche in the prostate. **Results**: E2-stimulated SCCM assays indicate that only high concentrations of SCCM can significantly alter expression of stemness genes in PS. Data from BrdU assays show that co-culture of PS with PrSCs significantly increases the number of BrdU label-retaining cells per PS, however, no significant estrogenic effect was seen. Furthermore, co-culture with PrSCs resulted in a significant decrease in gene expression of the luminal epithelial marker cytokeratin 8, an effect that was also independent of E2. Comparison of gene expression profiles between CAFs and PrSCs suggests that CAFs overexpress genes involved in stem-cell modulating pathways. **Conclusion**: Using primary human prostate cells, we conclude that normal stromal cells secrete paracrine factors that can modulate self-renewal of epithelial stem cells, however, the role of E2 in regulating these interactions is inconsistent. Gene expression profiling of normal PrSCs and CAFs shows that CAFs have a large number of differentially regulated genes, many of which are involved in putative E2-regulated and stem cell modulating pathways. Therefore, we will focus future efforts on elucidating how E2 might influence CAF paracrine signaling within the stem cell niche.

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Cell-Intrinsic Factors Influence Mechanisms of CD4+ T Cell Interstitial Motility in the Inflamed Dermis

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The effector function of $CD4^+$ T cells is critically dependent on their ability to rapidly enter and survey peripheral tissues for damage or infection. Although the processes of tissue homing and exit from the vasculature during inflammation have been well-studied, it remains unclear how $CD4^+$ T cells migrate through interstitial spaces following inflammatory insults. Changes to the structure of the extracellular matrix, the type and activation status of tissue resident immune cells, and the local balance of inflammatory chemokines and cytokines may all shape the behavior of infiltrating $CD4^+$ T cells. Through intravital multiphoton microscopy, we have recently demonstrated that Th1 cells require the integrin $alpha_V$ for migration along collagen fibers in the dermis following immunization with complete Freund's adjuvant (CFA), a strong pro-inflammatory stimulus. Th2 cells in the same environment show gross similarities in movement in the CFA-immunized dermis, moving with the same average velocity and directionality in an $alpha_V$-dependent manner. However, a fine analysis of motility reveals that Th1 and Th2 cells have altered patterns of movement within the same tissue. Th1 cells have a significantly higher arrest coefficient than Th2 cells, possibly indicating an enhanced ability to interact with resident antigen presenting cells or a differential response to the local chemokine and cytokine milieu. To further dissect the contributions from exogenous chemokine signals, we treated mice systemically with pertussis toxin (PTx) to block signaling through $G\text{-}alpha_i$, an intermediate in chemokine receptor signaling. Strikingly, the migration of Th1 cells is critically dependent on signals through $G\text{-}alpha_i$, with cells arresting and losing polarity following PTx treatment. However, Th2 cells remain insensitive to PTx blockade and retain both polarity and directed movement through the dermis, suggesting that Th2 cells migrate independently of exogenous chemokine signals. Interestingly, the movement of Th2 cells following PTx treatment remains $alpha_V$ integrin-dependent, suggesting that integrin activation in this setting is independent of chemokine signals. Studies are ongoing to determine potential differences in downstream chemokine and integrin signals in both subsets, as well as to characterize the local chemokine and cytokine milieu at the RNA and protein level. These fundamental differences in the migration of $CD4^+$ T cell subsets may reveal targets for the development of cell-specific immunomodulatory therapy to mitigate distinct immune pathologies.

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Divergent Effects of Long-Term Intermittent or Sustained Hypoxia on Brown Adipose Tissue

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Background: Brown adipose tissue (BAT) has recently gained substantial attention by virtue of its role in increasing caloric expenditure and promoting resistance to metabolic syndrome and obesity. Mitochondrial amount and activity are one of the key mechanisms by which BAT induces thermogenesis. Sympathetic stimulation, such as cold exposure or β-agonists, increases the BAT size, and yet, short term hypoxia, a powerful sympathetic stimulus, inhibits BAT thermogenesis. In the current study we aimed to dissect the differential effect of prolonged sustained hypoxia (SH), such as occurs in high-altitude dwellers, and of long term intermittent hypoxia (LT-IH), a condition closely mimicking patients with sleep apnea, on the transcriptional profile of mitochondrial and brown fat genes in BAT. **Methods**: 8-week old male C57BL/6J mice (n=10-12/group) were exposed to LT-IH (F_IO_2 6.1% alternating 21% every 90s, mean F_IO_2~8% for 12-hr/day during the light period) or to room air (RA) for 20 weeks and were kept on a regular low-fat chow. Another group of mice was exposed to continuous sustained hypoxia (SH; F_IO_2=8%) or RA for 5 weeks (n=5-6/group). Following exposures, intra-scapular BAT were excised and analyzed for gene expression using qPCR. **Results:** BAT mass was increased in SH but was significantly decreased in LT-IH when compared to RA (3.5 *vs* 1.4 *vs* 1.9 mg/g body weight respectively; $p<0.01$). ASC-1, a marker of white adipose tissue, and UCP-2, a white adipocyte specific mitochondrial uncoupling gene, were significantly increased in SH, but not in LT-IH. Conversely, BAT specific markers PX2R5 and UCP-1 were decreased in both experimental groups, with SH exhibiting a more pronounced effect. When expression of genes activated in response to cold or sympathetic stimuli, namely PGC1-α and its downstream target TFAM (inducer of mitochondrial DNA translation) were assessed, SH had no effect on either. In contrast, LT-IH induced significant up-regulation of PGC1-α (≈3.5 fold), while TFAM was markedly reduced. Finally, VEGF down-regulation, previously associated with vascular rarefaction and BAT dysfunction, was apparent only in the LT-IH group. **Conclusions**: Both hypoxic stimuli, SH and LT-IH, induced down-regulation of BAT markers. However, while SH leads to compensatory expansion of white adipocyte populations within BAT and potentially reduced thermogenesis, LT-IH induces lipolysis of BAT along with decreased angiogenesis and mitochondrial dysfunction. We hypothesize that the discrepant effects of these 2 hypoxic exposures may causally relate to the excessive reactive oxygen species generation in LT-IH and the potentially different transcriptional networks activated by LT-IH and SH, ultimately promoting divergent metabolic profiles. We are currently testing this hypothesis.

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Manipulating Host Signaling for Pathogenic Purposes: How and Why Kaposi's Sarcoma-Associated Herpesvirus Induces the Transcription Factor Nrf2

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Kaposi's sarcoma-associated herpesvirus (KSHV) is the etiological agent of two highly aggressive AIDS-related malignancies, endothelium-derived Kaposi's sarcoma (KS) and B cell-derived primary effusion lymphoma (PEL). Although current treatment methods have decreased the overall morbidity and mortality in immunocompromised and AIDS patients, KS and PEL remain difficult to treat. Thus, novel therapeutic methods are essential to significantly improve the life expectancy in PEL patients and quality of life in KS patients. One of the signaling pathways that is induced by KSHV infection are reactive oxygen species (ROS), which are well-known inducers of the transcription factor Nrf2. Because Nrf2 plays an important role in the biology of several viral infections, and is an important agent in cancer progression, we recently undertook a series of studies to fully investigate its role in KSHV infection of endothelial and B cells. Here, we show that KS lesions exhibit elevated levels of phosphorylated, active Nrf2. Using *de novo* infection of primary endothelial cells a model for KS, and latently-infected B cell lines as models for PEL, we further confirmed that KSHV induces Nrf2 stability, phosphorylation and nuclear translocation. We show that KSHV infection drives Nrf2 activity through ROS elevation during early infection and through the COX-2/PGE2 inflammatory axis during latency. We found Nrf2 important for VEGF-A, Bcl-2, and pentose phosphate pathway enzymes induction during *de novo* KSHV infection. More significantly, Nrf2 induction was necessary for optimal COX-2 expression during KSHV infection, establishing a feed-forward loop between these two promiscuous agents. We further knocked down Nrf2 in endothelial and B cells by lentivirally transducing shRNA against the Nrf2 transcript. Through RNA deep sequencing analysis we observed that Nrf2 knockdown significantly affected the expression profile of the KSHV genome. Real-time PCR analysis of several genes confirmed the deep sequencing findings, where we further found that the expression ORF50 and ORF73, two key regulators of the KSHV life cycle, were significantly altered by Nrf2 knockdown. Using the bioimaging technique proximity ligation assay (PLA), we determined that Nrf2 and the viral latency-associated nuclear antigen 1 (LANA-1), the protein product of ORF73, colocalize on the KSHV genome. Subsequent chromatin immunoprecipitation analysis determined that Nrf2 and LANA-1 co-occupy the promoter region of both ORF73 and ORF50, likely modulating their transcription. Collectively, our studies argue for a crucial role of Nrf2 during KSHV infection and pathogenesis.

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Diabetes-Associated Mutations in Human Proinsulin: Design, Synthesis, and Characterization of Single-Disulfide Peptide Models of an On-Pathway Folding Intermediate

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Introduction: Human metabolic homeostasis requires regulated secretion of insulin, a small two-chain globular protein hormone secreted by pancreatic β-cells. The mutant proinsulin syndrome, a monogenic cause of diabetes mellitus, results from dominant mutations in the insulin gene whose expression reduces the foldability of single-chain precursor proinsulin. Toxic misfolding of the variant proinsulin in β-cells impairs viability through aberrant protein aggregation in the endoplasmic reticulum. Such mutations identify key determinants of proinsulin folding efficiency. Of particular interest, non-cysteine-related substitutions in the B-domain of proinsulin give rise to a spectrum of disease severity and age of onset. Our studies focus on one mutation with neonatal onset (Tyr^{B16}->Pro) and another with delayed onset in early adulthood (Phe^{B24}->Ser). We hypothesized that respective ages of disease onset would correlate with extent of biophysical perturbation on the structure and stability of an on-pathway oxidative protein-folding intermediate. **Methods:** Experimental design exploited a novel 49-residue single-chain polypeptide containing only one disulfide linkage step as a model of the first on-pathway oxidative folding intermediate. The 49-residue polypeptide was based on synthetic intermediate *des*-Di (Zaykov AN, Mayer JP, Gelfanov VM, & DiMarchi RD. *ACS Chem. Biol.* (2014) 9, 683-91). Products were obtained by solid-phase peptide synthesis and purified by semi-preparative reverse-phase HPLC. In our model cysteine A7-B7 was pairwise substituted by Ser whereas cysteine A6-A11 was pairwise substituted by Ala. To enhance nascent structure and solubility at neutral pH, the wild-type (WT) or variant polypeptides (Pro^{B16} or Ser^{B24}) contained additional substitutions His^{B10}->Asp and Thr^{A8}->Glu. The three synthetic products were respectively designated 1SS-WT, 1SS-Pro, and 1SS-Ser. Circular dichroism (CD) and 2D nuclear magnetic resonance (NMR) were employed as biophysical probes. **Results:** 1SS-WT and 1SS-Ser exhibit partial folds. CD studies demonstrated a decrease in α-helix content of 1SS-WT relative to the native state of human insulin (or *des*-Di); a further loss of helix content was observed in 1SS-Ser. CD spectra of 1SS-Pro by contrast indicated a disordered polypeptide. 2D-NMR studies demonstrated a corresponding trend in extent (or attenuation) of chemical-shift dispersion. 2D TOCSY and NOESY experiments are in progress to delineate regions of nascent structure in 1SS-WT and their partial or marked destabilization in 1SS-Ser and 1SS-Pro, respectively. **Conclusion:** Our findings provide insight into the mechanism of toxic misfolding of proinsulin in the mutant proinsulin syndrome. Trends observed in CD and NMR studies support the hypothesis that the severity of disease phenotype (*i.e.*, neonatal vs. delayed adult-onset) correlates with degree of perturbation in the nascent structure of an on-pathway folding intermediate.

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Expression of microRNA-17 is Increased Within the Intestinal Epithelium in Premature Infants with Necrotizing Enterocolitis

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Background: Necrotizing enterocolitis (NEC) is the leading cause of gastrointestinal death in preterm infants. One of the challenges in NEC research is the ability to determine which babies are at risk for NEC development. While regulation of inflammatory gene expression within the intestine is not well understood, recent studies demonstrate that small noncoding RNAs called microRNAs (miRNAs) can regulate intestinal barrier function and the inflammatory immune response. The microRNA 17~92 cluster, which includes microRNAs 17, 18a, 19b, and 20a, has been linked with diseases involving intestinal inflammation, including inflammatory bowel disease, however the association between microRNAs and NEC remains unexplored. **Objective:** We hypothesized that microRNAs, specifically those in the miRNA-17~92 cluster, are differentially expressed in the intestine of premature infants with NEC and that certain miRNAs play a critical role in the regulation of the intestinal barrier dysfunction seen in NEC. **Design/Methods:** This study was performed after approval by the University of Pittsburgh Institutional Review Board protocol number PRO09110437. Intestinal samples were obtained from human neonates undergoing intestinal resection for NEC or at the time of stoma closure ("healed NEC"). RNA was isolated from these samples using TRIzol® Reagent (Ambion®). A total of 10ng/µl RNA was reverse transcribed to cDNA using TaqMan MicroRNA Assays (Applied Biosystems) with RT primers specific for the miRNA of interest, and PCR amplification reactions were ran on a C1000 Touch Thermal Cycler System (BioRad, Inc.). **Results:** Intestinal samples from premature infants with NEC demonstrated a statistically significant increase (p=0.0017) in expression of microRNA-17, versus intestine resected at the time of stoma closure. The other microRNAs in the 17~92 cluster, did not show a statistically significant difference in NEC versus healed NEC tissue. **Conclusions:** Intestinal expression of microRNA-17 was significantly increased in premature infants with surgical NEC. The identification of novel biomarkers, such as miRNA expression, may raise the possibility for novel diagnostic and therapeutic approaches of this devastating disease by further exploring microRNA expression in other biological samples from affected infants.

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Gray and White Matter Brain Metabolomic Profiling in a Porcine Model of Stroke with Human Induced Neural Stem Cell Treatment

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Stroke is a leading cause of death and severe long term disability in the United States. Regenerative treatments are needed to lessen the burden of the disease in surviving individuals. Neural stem cells (iNSCs) derived from induced pluripotent stem cells are a promising option with their ability to potentially replace lost neural cells and secrete anti-inflammatory and pro-survival paracrine factors. Hundreds of pharmaceutical treatments targeting stroke have advanced far in animal models, but failed in human clinical trials. One reason for this translational failure may be the discrepancy between small rodent and human brain anatomy. Stroke pathology is complex and heterogeneous, with multiple subtypes affecting gray and white matter neural tissue. Human brains have >60% white matter, while rodents have <10%. Pigs (Sus scrofa) have a large, gyrencephalic brain much more similar to humans than rodents, with more than 60% white matter. In order to better characterize and proof stem cell therapies for human patient treatment, we propose to use this large brain stroke model to study the effects of stoke in gray and white matter and the ability of stem cell treatment to reverse this damage. We used a metabolomics approach to study the biochemical phenotype of systemic and brain tissue metabolite changes after stroke and stem cell treatment. Metabolomic profiling uses advanced analytical techniques such as gas chromatography-mass spectroscopy (GC-MS) to profile changes of hundreds of endogenous molecules including amino-acids, sugars, lipids, neurotransmitters and their metabolic intermediates. Metabolite profiles were generated from serum and gray and white brain tissue samples collected 1 from control and stoked pigs with and without human iPSC derived NSC treatment using GC/MS profiling. Multivariate statistical analysis showed marked separation of white and gray matter samples. Differences in metabolic profile are also seen between treated and untreated groups. Using metabolomic profiling we show that significant differences in response to stroke injury and treatment are seen in gray and white matter within our porcine stoke model. These data and techniques will help to elucidate the pathological effects of stroke in gray and white matter and will help to evaluate stem cell treatments for ischemic neural injury.

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Non-Neuromuscular Manifestations of Spinal and Bulbar Muscular Atrophy

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Objective: To report non-neuromuscular manifestations in patients with spinal and bulbar muscular atrophy that have not been previously investigated. **Background:** SBMA is a neuromuscular disease caused by repeat expansion in the androgen receptor (AR) gene. SBMA is characterized by atrophy, fasciculations, and weakness in the limb and bulbar muscles starting in early adulthood. Females are asymptomatic or mildly symptomatic. Although neuromuscular findings and androgen insensitivity of SBMA are well known, there are other features of the disease that have not been well described. Our group reports on novel, non-neuromuscular characteristics in SBMA—liver function, serum growth factor levels, mood, and fatigue. **Design/Methods:** We assessed a group of fifty subjects ages 29 to 74 with spinal and bulbar muscular atrophy at the National Institutes of Health Clinical Center. Evaluations included assessments of muscle strength and function via Adult Myopathy Assessment Tool and Quantitative Muscle Assessment, biochemical laboratory analysis, quality of life via a self-reporting survey (Short Form 36 Health Survey Version 2) and mood assessment surveys (Beck Depression Inventory-II and the Aggression Questionnaire). The vitality index used in the SF-36v2 survey is widely regarded as an acceptable measure of fatigue. We completed liver examinations on a subset of 20 of the 50 subjects including laboratory analysis, hepatology consultation, liver imaging using magnetic resonance imaging with spectroscopy, and analysis of iPSC derived hepatocytes. **Results:** Insulin-like growth factor 1 (IGF-1), a hormone mostly synthesized in the liver, was found to be below 1 standard deviation from age-corrected levels in 32/50 (64%) of patients with a mean value of 147 ng/ml (87-283 ng/ml). The SBMA subjects were found to have low vitality scores with an average score of 44.5 as compared to control measurements (P=0.05). We observed evidence of hepatic steatosis in 90% of the subjects evaluated. RNAseq analysis of iPSC derived hepatocytes was performed. **Conclusions:** These non-neuromuscular observations provide new insight into spinal and bulbar muscular atrophy and offer additional avenues for investigating the disease mechanism.

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Medical Student Inter-Observer Variability Versus Automated Variability in Contouring Intracranial Normal Tissue: Implications for Radiation Therapy

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Background: Radiation Oncologists design treatments by targeting tumor volumes and avoiding nearby normal structures. Excess radiation to normal tissue, or organs at risk (OARs), leads to long-term damage potentially avoidable with improved treatment planning techniques. Normal tissue radiation varies by plan with necessity of hand-drawn contours for certain OARs. These manual contours are also time consuming due to total sum of OARs, creating the need for an efficient and precise solution. While computer-generated auto-contours have helped reduce inter-observer variability, some time spent editing computer contours is required. Manual contours, even inexperienced ones, may be better than computer contours. This project aims to determine whether computer auto-contours are superior to medical student manual contours for discerning certain Head and Neck structures. **Method:** Seventeen consecutive Head and Neck cancer patient scans from the past two years were selected from Radiation Oncology MOSAIQ database in this IRB approved study. T1-weighted, contrast enhanced MRI axial scans were imported into the BrainLAB treatment planning software (TPS) and contoured by five users. Window/level setting for all contours was kept fixed at 200 - 800. Head and Neck OARs selected in this study represented varying degrees of radiation sensitivity: Brainstem, Cerebellum, Optic Chiasm, Eyes, Lenses, Optic Nerves, and Optic Tracts. User 1, a faculty medical physicist, contoured the reference structures. Three medical students (Users 2-4) drew contours to measure manual-user variability. Computer contours were represented as User 5. Common intersection points were determined between all contours and their corresponding reference structures, and contour variability was determined using Overlap Index (OI) and Dice Similarity Coefficient (DSC). Degree of overlap was determined based on a 0 to 1 scale, with 1 being perfect overlap. Two-tailed Student T-tests were performed to determine statistically significant differences. **Results:** Student group had a significantly greater degree of overlap with reference contours for Brainstem, Cerebellum, Eyes, and Optic Nerves ($p < 0.05$). This was true for both OI and DSC parameters of comparison. Based on DSC, the Right Lens also had significantly greater overlap in the student group. Data was trending towards greater overlap for both Optic Tracts by the automatic group but this trend was not statistically significant ($p > 0.05$). The Optic Chiasm showed no preference in overlap between groups. **Conclusion:** Computer auto-contours may improve efficiency of treatment planning, but still exhibit marked variation from standard reference contours. Student manual contours exhibit some variation but were found to be better than automatic contours for certain Head and Neck structures.

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Breast and Cervical Cancer Screenings: A Comparison Between Latinas in Wisconsin and California

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Background: Foreign-born Latinas have higher rates of never receiving breast or cervical cancer screening, compared to U.S.-born Latinas and non-Latina Whites. The purpose of this study is two-fold. First, we compare the rates of last 12-month breast and cervical cancer screening among four populations of Latinas representing different community contexts, levels of acculturation, and migration status. Second, we identify factors contributing to the utilization of screening among Latinas, including sociodemographic and migration factors, health insurance status, and availability of regular health care provider. **Methods:** We used data from a random cross-sectional sample of Latinas who participated in a phone survey in Dane County, WI between 2009 and 2010, including and a cross-sectional probability survey of migrants returning to Tijuana, Mexico from California in 2013. Four groups of Latinas were differentiated: (1) U.S.-born Latinas residing in Wisconsin (USWI, N=60); (2) Mexico-born Latinas residing in Wisconsin (MXWI, N=168); Mexico-born Latinas returning to Mexico from California via deportation, a proxy for unauthorized migration status (MXCADEP, N=61); and (4) Mexico-born Latinas returning from California to Mexico voluntarily, a proxy for authorized migration status (MXCAVOL, N=184). Descriptive statistics and multivariate logistic regressions were estimated. **Results:** The rate of last 12-month breast cancer screening was highest among MXCAVOL (40.2%), followed by MXWI (25.6%), U.S.-born (23.3%), and MXCADEP (23.0%; p<0.01). In contrast, the rate of last 12-month cervical cancer screening was highest among MXWI (64.3%) compared to MXCAVOL (48.9%), USWI (46.7%), and MXCADEP (18.0%; p<0.001). The four groups differed significantly in levels of education, health insurance status, availability of a regular health care provider, and length of residence in the U.S. (p<0.01). After adjusting for socioeconomic and health care access factors, MXCADEP (OR= 7.04, 95% CI: 1.88-26.3), MXCAVOL (OR=3.75, 95% CI: 1.59-8.83), and MXWI OR=2.77, 95% CI: 1.13-6.76) were significantly more likely to receive a mammogram than USWI. Compared to USWI, MXCADEP were less likely to report receipt of a Pap smear (OR=0.24, 95% CI: 0.07-0.82). Being single was also found to be significantly associated with receipt of Pap smear (OR=0.52, 95% CI: 0.33-0.81), whereas having health insurance (OR=2.47, 95% CI: 1.37-4.47) and availability of regular healthcare provider (OR=2.34, 95% CI: 1.21-4.51) were found to be associated with mammogram receipt. **Conclusion:** Acculturation level, regional contexts, and migration status contribute to suboptimal rates of mammogram and Pap smear receipt among Latinas residing in Wisconsin and those in California. Public health programs addressing these factors are needed to promote breast and cervical cancer screenings among these underserved populations.

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Identification of Novel Genetic Drivers of Vulvar Carcinoma in a Sleeping Beauty Model of Spontaneous Cancer

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Introduction: The purpose of this study was to develop a Sleeping Beauty (SB)-induced mouse model of metastatic vulvar carcinoma with which we could identify novel tumor-driving mutations. **Experimental Procedures:** An existing transgenic mouse model known to induce development of benign vaginal papillomas, ($Pr^{Cre/+}$;$Kras^{G12D}$), was bred to mice containing the components of the SB system to render ($Pr^{Cre/+}$;$Kras^{G12D}$;*SB*) mice. The SB system consists of a transposable element (transposon) and an activating enzyme (transposase) that induces excision of the transposon from one site to another in the host cell genome. Insertional mutations that enhance cell survival and proliferation are selected for, resulting in the development of cancer. We utilize the SB system to identify candidate driver mutations since the transposon is not only the mutagen but also serves as a sequence tag for localization of mutations. Mice were aged and monitored for phenotypic signs of disease. Upon necropsy, gynecologic and other grossly abnormal organs were harvested. Genomic DNA was extracted from each sample, prepared for genetic analysis, and sequenced using the Illumina platform. Candidate genes were identified using an unbiased Monte-Carlo approach in conjunction with a gene-centric Chi-Squared analysis. Paraffin-embedded human tumor samples were obtained from the University of Iowa Hospitals and Clinics for immunohistochemical labeling of protein expression for selected genes. **Results:** Vulvar carcinoma developed in 29 of 29 $Pr^{Cre/+}$;$Kras^{G12D}$;SB female mice with metastases by seven months of age confirmed in 13 animals. Organs within the abdominal cavity including the uterus and ovaries contained metastatic lesions in some mice. In one mouse, a distant metastasis within the subcutaneous tissue of the thoracic back was observed. Illumina sequencing of primary lesions from all mice revealed 77 candidate tumor drivers. **Conclusions:** To our knowledge, the $Pr^{Cre/+}$;$Kras^{G12D}$;SB mouse is the first reported metastatic model of vulvar carcinoma. Protein expression of candidate genes identified by our SB screen are being assessed in human tissue samples. Patient clinical data will be incorporated into analyses to determine whether candidate genes are correlated to patient outcomes. This work will permit insights into the pathophysiology and future treatment of vulvar cancer.

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Heterozygous Mutations in Aggrecan Cause Short Stature, Accelerated Bone Maturation, and Early Growth Cessation

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Short stature is a common presentation to pediatric endocrinology clinics and is frequently associated with delayed skeletal maturation. In contrast, short stature with advanced skeletal maturation is a rare presentation. We recruited four families with autosomal dominantly inherited short stature and advanced skeletal maturation. Affected family members presented with childhood short stature (height SDS -1.9 to -3.5), advanced bone age, early growth cessation, and adult short stature (height SDS -2.6 to -4.7). Additional features that were variably present include osteochondritis dissecans, early onset osteoarthritis, macrocephaly, midface hypoplasia, and exaggerated lumbar lordosis. To identify the genetic cause of this phenotype, we performed whole exome sequencing in selected individuals from each family. In all four families, we identified novel heterozygous variants in the aggrecan gene (*ACAN*), which encodes a proteoglycan that serves as a major structural component of the extracellular matrix in growth plate and other cartilaginous tissues. The sequence variants were present in all affected but in none of the unaffected family members. Each of the mutations identified was predicted to result in loss of protein function. Our study indicates that heterozygous mutations in *ACAN* cause a mild skeletal dysplasia that presents as short stature with advanced bone age and early growth cessation. This contrasts with previous reports of *ACAN* mutations identified in individuals with severe skeletal dysplasias. Our findings thus expand the spectrum of *ACAN* defects and provide a new molecular genetic etiology for the unusual child who presents with short stature and accelerated skeletal maturation.

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A Novel Anti-Apoptotic Role of ABCB5 in Cancer Stem Cells

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Cancer stem cells (CSC) responsible for disease progression and therapeutic resistance have been identified in several human malignancies, including colorectal cancer (CRC). However, the molecular mechanisms through which CSC drive tumor growth are incompletely understood. ABCB5, a member of the ATP-binding cassette superfamily of active transporters, serves as a CSC-specific multidrug resistance mechanism in diverse human malignancies. Additionally, ABCB5 has recently been demonstrated to function as an anti-apoptotic gene in tissue-specific non-malignant stem cells (Ksander et al., Nature, 2014). Here we demonstrate that ABCB5 also serves an anti-apoptotic role required for CSC maintenance in human cancer. Targeted inhibition of ABCB5, previously shown to be preferentially expressed on CD133-positive CRC stem cells, induced tumor cell apoptosis *in vitro* and *in vivo* and inhibited human CRC growth in NSG recipient mice. Mechanistically, ABCB5-positive tumor cell ablation through monoclonal antibody-mediated blockade or shRNA-mediated gene knockdown resulted in diminished production of the receptor tyrosine kinase AXL, a pro-tumorigenic molecule identified herein to be preferentially produced by CRC stem cells. Restoration of AXL expression through gene transfection in ABCB5 knockdown tumors partially restored tumor growth, demonstrating that ABCB5-positive CRC stem cells drive tumorigenicity at least in part through production of AXL. Our results establish a novel anti-apoptotic function of ABCB5 in human cancer and indicate that targeted blockade of ABCB5 represents a novel strategy for CSC eradication, independent of its previously established function as a multidrug resistance mediator.

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Targeted Transplantation of Mitochondria to Liver Cells

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Background and Aim: Genetic and acquired defects in mitochondria can result in severe liver damage and dysfunction causing disease and death. Mammalian hepatocytes possess specific receptors on their surfaces, asialoglycoprotein receptors (AsGRs), which can recognize and bind asialoglycoproteins (AsGs). After binding, AsGs are internalized by receptor-mediated endocytosis. The aim of this study was to determine whether mitochondria could be targeted specifically to hepatocytes and taken up by the AsGR pathway. **Methods:** An asialoglycoprotein, asialo-orosomucoid (AsOR), was fluorescently labeled to make Fl-AsOR which was covalently linked to polylysine to create a positively charged conjugate Fl-AsOR-PL capable of binding to mitochondria which are negatively charged. GFP-labeled rat mitochondria were isolated from HTC mito-GFP cells and mixed with Fl-AsOR-PL to form stable electrostatically bound complexes. To assess targeted delivery of mitochondria to hepatocytes, Fl-AsOR-PL-mitochondria complexes were incubated separately with Huh7 AsG receptor (+), and SK Hep1 cells AsG receptor (-) human hepatoma cell lines at 37°C, and cells sampled at various time points. After extensive washing, intracellular uptake of rat mitochondria was assayed by qPCR using primers specific for rat mitochondrial DNA, and dual photon confocal fluorescence microscopy to detect GFP, and anti-EEA antibody followed by Alexafluor 594 to detect early endosomes. **Results**: Fluorescence data showed that the Fl-AsOR-PL conjugate remained stably bound to mitochondria after multiple spin and re-suspension cycles. Incubation of Fl-AsOR-PL-mitochondria complexes with cells showed that only Huh7, but not SK Hep1 cells had significant Fl-AsOR fluorescence, and that fluorescence increased with time. qPCR confirmed that rat mitochondrial DNA increased with time in Huh7, but not SK Hep1 cells. Incubation of Fl-AsOR-PL-mitochondria complexes with Huh7 cells in the presence of a large molar excess of free AsOR blocked the association of fluorescence with those cells. Confocal microscopy confirmed the presence of intracellular mitochondria. Overlapping GFP and Alexafluor 594 indicated the presence of Fl-AsOR-PL-mitochondria in endosomes. Co-targeting of an endosomolytic agent confirmed initial co-localization of mitochondria in endosomes, as well as intracellular rat mitochondria unassociated with endosomes. **Conclusions:** Coupling of polylysine to AsOR results in a strongly positively charged conjugate, Fl-AsOR-PL that bound mitochondria in a stable non-covalent interaction. Fl-AsOR-PL-mitochondria complexes were taken up by hepatocytes by receptor-mediated endocytosis, and levels of endocytosed complexes increased with time. Co-targeting of an endosomolytic agent increased co-localization of mitochondria in endosomes, as well as intracellular rat mitochondria unassociated with endosomes. This is the first demonstration of targeted uptake of mitochondria in mammalian cells, hepatocytes, by receptor-mediated endocytosis.

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Identification of Novel Drug Targets in Mycobacterium Avium and Other Nontuberculous Mycobacteria

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Almost thirty years after the introduction of the extended spectrum macrolide antibiotics clarithromycin and azithromycin for treatment of *Mycobacterium avium-intracellulare* complex (MAC) infections, pharmaceutical treatment failure in both HIV negative and positive patient populations remains alarmingly high. The inherent resistance most MAC strains exhibit to anti-mycobacterial drugs, coupled with selected resistance to macrolide antibiotics, further complicates an already difficult treatment regime. Physicians are in desperate need for novel, potent antibiotics capable of achieving full pharmacological resolution of disease in patients with MAC pulmonary, soft tissue, and disseminated infections, yet the development of such new drugs has not occurred. Utilizing high throughput methodologies, we developed a whole cell toxicity assay and screened approximately 54,000 diverse compounds with drug-like properties against a macrolide resistant strain of *Mycobacterium avium*. Following validation of activity, confirmed compounds were assayed for minimal inhibitory concentration and minimal bactericidal concentration, as well as for toxicity in human fibroblasts and yeast. Whole cell active compounds were then prioritized for selection of resistant mutants, sequencing, and target identification. Validation of new targets is a crucial step in developing novel antimicrobials. The end goal of our investigation will be to expand known potential drug targets for mycobacterial pathogens and characterize basic structure-activity relationships between initial screen compounds with the hopes of developing potent lead compounds for these new targets in the future.

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Intermittent Hypoxia Induces TGF-β1-Associated Myofibrosis in Peripheral Artery Disease by Oxidative Stress Dependent Endothelial PDGF-BB Signaling

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Introduction: The gastrocnemius of Peripheral Artery Disease (PAD) patients experiences chronic intermittent hypoxia and exhibits progressive myofibrosis that is associated with increased transforming growth factor-beta 1 (TGF-β1) expression in smooth muscle cells (SMC) of oxidatively damaged vessels. PDGF-BB signaling between endothelia (EC) and SMC has been implicated as a mechanism by which TGF-β1 is increased in other fibrotic diseases. However, this mechanism and contributions of oxidative stress have not been assessed in PAD. We hypothesized that intermittent hypoxia oxidatively damages EC to increase PDGF-BB expression that induces SMC expression of TGF-β1. **Methods:** We used a novel method Quantitative Fluorescence Imaging Analysis (QFIA) to determine the correlation between expression of vascular PDGF-BB and TGF-β1, and levels of oxidative damage markers, 4-hydroxynonenal (4-HNE) and carbonyl, in tissue sections of gastrocnemius from PAD (n=20) and Control (CTRL, n=10) patients. Immunofluorescence was used to co-localize PDGF-BB, TGF-β1, carbonyl, and 4-HNE to EC (CD31) and SMC (high-molecular-weight caldesmon). *In vitro*, primary human umbilical vein endothelial cells (HUVEC) were exposed to 5 days of either normoxia (20% O_2 and 5% CO_2) or intermittent hypoxia (1.5 hours normoxia, 1.5 hours of 1.5% O_2 and 5% CO_2) in an OxyCycler42 computer-controlled system that simultaneously regulates O_2 and CO_2 tension. At the end of 5 days, PDGF-BB, 4-HNE, and carbonyl were measured by Western Blot. Contribution of oxidative stress was determined by adding 100uM of Apocynin or MitoTempo at Days 1 and 3. Supernatant from hypoxic HUVEC, either treated for 1 hour with PDGF-BB neutralizing antibodies or saline, was transferred to primary human internal thoracic artery smooth muscle cell (HITASMC) and expression of TGF-β1 measured by ELISA after 3 days. **Results:** PDGF-BB increased in PAD compared to CTRL patients ($p<0.001$), primarily localized to EC, and correlated with TGF-β1 expression ($r=0.741$, $p<0.01$). Total vascular 4-HNE and carbonyl damage was increased in PAD compared to CTRL patients (both $p<0.01$), but only 4-HNE robustly localized to EC. In hypoxic compared to normoxic HUVEC, PDGF-BB expression ($n=3$, $p=0.03$) and 4-HNE ($n=3$, $p<0.001$) but not carbonyl damage was increased, and were reduced with Apocynin ($n=3$, $p<0.001$) and MitoTempo ($n=3$, $p<0.01$). PDGF-BB neutralizing antibody treatment of supernatant significantly reduced the TGF-β1 increase in SMC that resulted from saline-treated supernatant ($n=3$, $p<0.001$). **Conclusions:** Endothelial production of PDGF-BB, in response to intermittent hypoxia, is oxidative stress dependent and induces SMC expression of TGF-β1. This mechanism is an important therapeutic target to interdict progression of TGF-β1 associated myofibrosis that likely contributes to limb dysfunction in PAD patients.

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Deciphering the Activation Mechanism of the Orphan Adhesion GPCR GPR110

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Introduction: The activation mechanism of Adhesion G Protein Coupled Receptors (aGPCRs) is currently unknown. aGPCRs undergo autoproteolytic cleavage at a conserved site creating receptors with two protomers. The extracellular domain (ECD) remains non-covalently bound to the 7-transmembrane (7TM) domain, an association thought to suppress G protein signaling. Ligand-mediated ECD removal is proposed to activate signaling. We hypothesize that the orphan aGPCR GPR110 contains a cryptic tethered agonist that activates the 7TM domain when it is revealed by removal of the ECD. This mechanism may be conserved among the 33-member aGPCR class. **Aims:** Determine the G protein coupling specificity of GPR110. Understand the mechanism of GPR110 and aGPCR activation. Identify synthetic peptides corresponding to the putative cryptic tethered agonist that modulate receptor activity. **Methods:** aGPCRs were expressed in insect cells using a baculovirus system. Prepared receptor membranes were treated with or without urea and used in heterotrimeric G protein biochemical reconstitution assays. Kinetic [^{35}S]-GTPγS binding measurements of GPR110-mediated G protein heterotrimer activation were made. Synthetic peptides were added *in trans* to study GPR110 pharmacology. **Results:** GPR110 couples to G_q and no other G protein classes (G_i, G_s, or G_{13}). ECD removal resulted in full G_q activation. Following ECD removal, the aliphatic stalk region located N-terminal to TM1 was exposed. Mutagenesis experiments showed that specific residues of the stalk were critical for GPR110-mediated G_q activation. A number of synthetic peptides from a series that comprised the stalk region antagonized constitutively active GPR110, whereas a single peptide acted as an agonist for both full-length (ECD-inhibited) GPR110 and receptors with mutagenized stalk regions. **Conclusions:** We have determined that a cryptic agonistic element of the GPR110 stalk region activates the GPR110 7TM protomer. We propose that the tethered agonist interacts with an orthosteric binding site of 7TM protomer to elicit a conformational change that facilitates receptor-mediated G_q activation. Parallel studies in the laboratory on the aGPCR GPR56 have generated strikingly similar results and support a conserved mechanism of aGPCR activation by a cryptic tethered agonist that is revealed by ECD removal.

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NALP3 Inflammasome Priming by Lipopolysaccharide is Inhibited by an F-Box Protein FBXO3 Antagonist

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Rationale: The inflammasome is a multi-protein complex that augments the pro-inflammatory response by increasing the release of key cytokines. Specifically, the NALP3 inflammasome requires two-step signaling, priming and activation, to be capable of releasing pro-inflammatory cytokines such as IL-1β and IL-18. These cytokines are associated with worse outcomes of the acute respiratory distress syndrome (ARDS). Through unknown mechanisms, the priming process increases the immunoreactive levels of NALP3 and pro-IL-1β in cells. We investigated how lipopolysaccharide (LPS) primes NALP3 levels in cells, and whether the release of cytokines can be modulated by altering the priming process. **Methods:** After treating human monocyte U937 cells with LPS, we measured protein levels of NALP3 by immunoblotting, and mRNA levels by real-time PCR after RNA isolation followed by reverse transcription. The half-life of NALP3 protein was measured by inhibiting new protein synthesis with cyclohexamide, both at baseline and after LPS exposure. The binding between two proteins were confirmed by co-immunoprecipitation (Co-IP). We precipitated extracellular media with trichloroacetic acid, and subsequently measured cytokine levels by immunoblotting. **Results:** We verified a dose- and time-dependent increase in NALP3 protein levels after LPS exposure in human monocyte cells. The steady-state mRNA expression of NALP3, however, was not changed after LPS exposure, suggesting post-translational regulation. The half-life of NALP3 is approximately 4 hours in an unchallenged condition, and prolonged to >6 hours after LPS exposure. NALP3 degradation was reduced with MG132, but not with a lysosomal inhibitor, leupeptin, indicating the degradation of NALP3 occurs via the ubiquitin proteasome system rather than the lysosome. NALP3-ubiquitin binding, measured by Co-IP, was decreased after LPS exposure. We found that ubiquitination of NALP3 is mediated by an E3 ligase component, FBXL2. The binding between NALP3 and FBXL2, measured by Co-IP, was also decreased after LPS exposure, as was NALP3 ubiquitination. A small molecule FBXO3 inhibitor, BC-1215, restores FBXL2 levels resulting in decreased NALP3 protein levels in cells and thereby reducing the release of IL-1β and IL-18 in human inflammatory cells. **Conclusions:** LPS increases NALP3 protein levels by decreasing its degradation via reduced ubiquitin-mediated proteasomal processing. We found that FBXL2, an E3 ligase component, mediates ubiquitination of NALP3, which is regulated by LPS. By targeting inflammasome priming, we were able to reduce the release of potent pro-inflammatory cytokines, IL-1β and IL-18. Our findings provide valuable mechanistic insights for inflammasome priming, which may serve in the future as a platform for preclinical interventions to improve the outcomes of ARDS patients.

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KRAS Transformed Pancreatic Epithelial Cells Undergo EMT in Response to TGF-b in the Context of Acinar-to-Ductal Metaplasia, Thereby Acquiring Stem-like Properties

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Pancreatic ductal adenocarcinoma (PDA) likely originates from acinar cells. To develop into PDA, acinar cells must undergo acinar-to-ductal metaplasia (ADM) to acquire ductal characteristics. The resulting metaplastic ductal cells are then thought to pass through a series of precursor lesions known as pancreatic intra-epithelial neoplasias (PanINs) before becoming PDA. A critical step in cancer development is epithelial-to-mesenchymal transition (EMT), whereby cancer cells penetrate the basement membrane and invade the surrounding stroma. This was initially thought to occur late in cancer development, but is know understood to be an early event, occurring during ADM and PanIN phases of cancer development. The various transitions (ADM, EMT, etc.) are thought to be the result of genetic mutations and environmental cues, but the details of this regulation are largely not understood. We have found using an in vitro 3D cell culture system that the cytokine TGF-b is able to elicit EMT in acinar cells that have undergone ADM and express oncogenic KRAS. EMT provides the cells with stem-like properties, including increased sphere forming efficiency, the ability to form orthotopic allografts, and expression of pancreatic cancer stem cell marker Dclk1. Interestingly, the EMT program in the these cells also seems to involve a liver reprogramming component, as EMT results in upregulation of many genes traditionally expressed by hepatocytes.

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Atrasentan, a Specific ET-A Blocker, Improves Podocyte Survival and Ameliorates Diabetic Glomerular Injury in the db/db Mouse

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Background: The endothelin (ET) system is chronically activated in diabetic nephropathy and retinopathy. We have shown that atrasentan administration to *db/db* mice ameliorates retinal vascular pathology while increasing the number of pericytes, a cell with similarities to podocytes. Here we report the effect of atrasentan on glomerular podocyte number and glomerular injury. **Methods**: Diabetic *db/db* mice were given either atrasentan or vehicle in drinking water for 8 weeks starting at 23 weeks of age. At the end of the treatment period (31 weeks of age), kidneys were harvested for WT-1 staining to perform podocyte count and for morphometric analysis (PAS staining). Mesangial matrix expansion, glomerular cellularity and glomerular size were evaluated in PAS stained sections. To examine the effect of atrasentan on podocyte apoptosis in hyperglycemic milieu, conditionally immortalized mouse podocytes were exposed to normal glucose (NG, 5mM), high glucose (HG, 25mM) and HG +

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Atrasentan(2uM), and caspase-3 activity was measured. **Results**: In atrasentan-treated *db/db* mice, glomerular mesangial matrix and glomerular cellularity decreased significantly as compared to vehicle-treated *db/db* mice(1.4 ± 0.2 vs 2.7 ± 0.3,respectively, p<0.001) . Glomerular size decreased in atrasentan-treated *db/db* mice (0.0048 ± 0.0002 vs 0.0031 ± 0.0001 mm^2, p <0.01). Atrasentan-treated diabetic *db/db* mice showed significantly higher podocyte counts per glomeruli as compared to vehicle-treated *db/db* mice (12.1± 0.4 vs 10 ± 0.4, respectively, p<0.01). Data from cultured podocytes showed that atrasentan significantly reduced glucose-induced apoptosis (as determined by reduction in caspase-3 activity) (22186 ± 4002 vs 14736 ± 2310 RFU, p< 0.05) suggesting it as a potential mechanism of increased podocyte survival. **Conclusions**: Atrasentan ameliorates glomerular diabetic lesions in the *db/db* mice and has a protective effect on podocytes, similar to our finding of increased retinal pericyte counts. The dual protective effect of atrasentan for diabetic retinopathy and nephropathy involves enhanced pericyte and podocyte survival.

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Effect of Bladder Distension on Dosimetry of Organs at Risk During Intracavitary Cervical HDR Brachytherapy Using Dose-Volume and Dose-Surface Histograms

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Purpose: During HDR brachytherapy of the cervix, the main organs at risk (OAR) for radiation exposure are the bladder, rectum, sigmoid colon and small bowel. The optimal treatment plan delivers the desired prescription dose to the target organ, while minimizing dose delivered to the OAR. Due to radiation intensity fall off, the position of the organs within the pelvic cavity affects the distribution of radiation dose. Bladder distension plays a significant role in this, as volume of the bladder can shift organs within the pelvic cavity. For thin-walled hollow organs, the surface area irradiated may give a more accurate measure of biological effect than full volume irradiated. The purpose of this study is to evaluate both dose-surface and dose-volume histograms to analyze the effect of filling the bladder during cervical brachytherapy. **Methods:** Twenty patient brachytherapy fractions were selected with institutional IRB approval. For each fraction, both empty and full (200 cc of contrast saline) bladder CT tandem and ovoid applicator scans existed, one of which had already been used to generate the patient's actual treatment plan. An identical plan was then created with the unused scan, yielding 20 full bladder treatments and 20 empty bladder treatments for this study. Varian Brachyvision was used to contour the bladder, rectum, sigmoid and small intestine. The dose-volume histogram (DVH) of each fraction was then recorded. Parameters included mean DVH dose and maximum 2cc dose to each OAR. Plans were then exported to Computational Environment for Radiation Research (CERR) to obtain dose-surface histogram (DSH) data. **Results:** Between full and empty bladder fractions, the dose to the bladder in full treatments showed no difference in maximum 2cc dose, but showed significantly reduced dose in the DVH and DSH. The sigmoid and small intestine showed significantly reduced dose in full bladder treatments in all DVH and DSH parameters. The rectum did not exhibit difference in any parameters when comparing treatments. **Conclusions:** Distending the bladder during brachytherapy significantly reduced dose in all DVH and DSH parameters for sigmoid and small intestine, likely due to the distended bladder pushing these organs away from the applicator. The bladder showed no difference in maximum dose received, but exhibited an overall reduction in radiation dose to the whole organ in cases with bladder distension. The reductions in dose to the bladder, sigmoid and small intestine indicate that filling the bladder when irradiating the cervix is a beneficial practice and has the potential to decrease incidence of radiation associated late complications.

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Investigations of the Multiple Effects of the Alpha2a-Adrenergic Agonist Guanfacine in the Bed Nucleus of the Stria Terminalis and its Relevance to Stress-Induced Reinstatement of Drug Seeking

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Among the 2.5 million people seeking treatment for addiction to alcohol or illicit drugs every year, approximately half will relapse with most citing stressors as the fundamental cause. Their experience has been modeled in rodents using a stress-induced reinstatement paradigm, which has shown that reinstatement is dependent on intact norepinephrine signaling within the extended amygdala. Specifically, direct administration of adrenergic modulators into the bed nucleus of the stria terminalis (BNST) reduces stress-induced relapse behavior. This preclinical finding has correlates with clinical findings, as addicted individuals report reduced cravings after treatment with the alpha2a-adrenergic receptor (alpha2a-AR) agonist guanfacine. Unfortunately, though, guanfacine-treated patients relapse at the same rate despite their reduced cravings. This finding could be due to conflicting actions of guanfacine on different subsets of alpha2a-ARs within the heterogeneous BNST, where guanfacine has the potential to both inhibit and enhance excitatory transmission. For example, guanfacine inhibits optically evoked glutamatergic transmission from the parabrachial nucleus to the BNST, but enhances the optically evoked field potential generated from excitation of non-parabrachial inputs to the region in a Thy1-COP4 transgenic mouse line. Current studies aim to decipher whether these two effects of guanfacine are complementary or in conflict with one another. Investigations into the effects of in vivo administration of guanfacine have shown that it leads to increased BNST expression of the immediate early gene c-fos, suggesting that the guanfacine enhancement of excitatory transmission leads to neuronal activation in this region. We hypothesize that this enhancement is occurring in a subset of BNST neurons characterized by the presence of a hyperpolarization-activated current via the inhibition of HCN channels downstream of the alpha2a-AR. Preliminary studies suggest that this is the case since the application of the HCN inhibitor ZD7288 recapitulates the enhancing effects

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of guanfacine on excitatory transmission in the Thy1-COP4 transgenic mouse line. This finding is consistent with studies showing that the effect of guanfacine in the prefrontal cortex also occurs via a similar mechanism. By better understanding the mechanisms by which guanfacine exerts its effects on stress-induced relapse in the extended amygdala, and how these effects might be at odds with one another, future therapeutics may be better tailored to maximize anti-addictive potential.

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How Does Type 2 Diabetes Impact the Cerebral Microvasculature in Transgenic Alzheimer's Disease Mice?

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Epidemiological data show that individuals with type 2 diabetes mellitus (DM2) are at an increased risk of developing sporadic Alzheimer's disease (AD), and autopsy analyses suggest that DM2-induced microvascular pathology mediates this risk. We used the PDAPP transgenic mouse model of AD in combination with a non-genetic DM2 model to investigate how DM2 impacts the neurovascular unit in AD. We divided PDAPP mice into three groups: a group fed a normal diet (ND), a group fed a high-fat diet and injected with streptozotocin (DM2), and a DM2 group that also received injections of 5A, a vasculoprotective apolipoprotein A1 mimetic peptide. Immunohistochemistry and/or Western blot revealed reduced aquaporin-4 and phospho-eNOS in DM2 group brains compared to ND group brains. These effects were rescued by 5A treatment. We used *in vivo* two photon microscopy to investigate other potential causes and consequences of neurovascular dysfunction. The number of leukocytes rolling along pial venules—a measure of vascular inflammation—showed a trend towards being elevated in the DM2 group compared to the ND group. The extent of β-amyloid plaque deposition in pial arterioles was not different between the three groups. However, we found a significant correlation between the number of rolling leukocytes and the vascular plaque burden. Treatment with 5A abolished this relationship by reducing leukocyte rolling without reducing plaque, suggesting that vascular inflammation is downstream of vascular plaque deposition. Finally, there was no difference in capillary blood cell velocity between anesthetized ND and DM2 animals. Altogether these data suggest that vascular amyloid increases vascular inflammation, which, under diabetogenic conditions, may trigger the dissociation of astrocytic endfeet from capillary walls and endothelial dysfunction. We will present measurements of capillary velocity in awake animals undergoing sensory stimulation, which may reveal if DM2 induces a functional derangement of the neurovascular unit. D.A.H. is supported by the MUSC MSTP through NIH T32 GM008716; N.R.B. is supported by ADDF grant #20131214.

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MicroRNA-206 Illuminating Differentiation Block and Potential Therapy for Pediatric Rhabdomyosarcoma

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Rhabdomyosarcoma (RMS) is the most common soft-tissue cancer in children and is a highly aggressive form of cancer. One-third of children with intermediate risk disease relapse after treatment and are likely to die of their malignancy. Children with high-risk disease suffer from a 3-year survival of only 20%. RMS tumors express early developmental skeletal muscle genes, but the origin and molecular underpinnings of this tumor are largely unknown. Despite the expression of muscle regulator proteins MyoD and myogenin, RMS tumors and cells fail to terminally differentiate suggesting that RMS is an arrested state of muscle development. Therapeutic agents to drive terminal differentiation and thus halt proliferation remain elusive. The fusion of the RD human RMS cell line to fibroblasts relieves the block allowing differentiation, thus suggesting that a regulator of the myogenic transcription factors is deficient in RD cells but is present in the heterocaryon. The ability to force RMS cells to differentiate suggests an opportunity for therapeutic intervention. Expression of muscle-specific microRNA-206 is induced during both primary myoblast and satellite cell differentiation. Interestingly, expression of miR-206 is lower in RMS than normal skeletal muscle and forced expression in RMS cell lines induces myogenic differentiation suggesting a potential therapeutic strategy. We have utilized our mouse model of RMS that activates a conditional oncogenic *Smoothened* allele, *SmoM2*, with an adipose protein 2 promoter-driven Cre recombinase allele, aP2-Cre. Similar to human RMS the aP2-Cre;SmoM2 tumor overexpressed early muscle development genes and displayed low expression of markers of terminal muscle differentiation. The miR-206 knockout mouse is a viable, fertile and has been bred into the aP2-Cre;SmoM2 mice and illustrates miR-206 deletion decreased the latency and increases the penetrance of tumor formation, suggesting miR-206 functions as a tumor suppressor in RMS. The identification of miR-206 as a tumor suppressor suggests that miR-206 replacement maybe of therapeutic benefit; however, the miR-206 constellation of target genes in RMS remain elusive. We overexpressed miR-206 in RD cells using a mimic nucleotide and performed have identified miR-206 target genes with a combination of gene expression, proteomic profiling with mass spectrometry as well as RNA immunoprecipitation followed by sequencing. These methods have identified regulators of cell cycle (*CDK4, CDK6, CCND1* and *CCND2)* and muscle differentiation (*PAX3, PAX7, MET*) as miR-206 target genes. Together this work will provide further insight into the mechanism by which miR-206 promotes rhabdomyosarcoma differentiation and determine the potential of miR-206 replacement for therapeutic intervention in RMS.

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Impact of Preoperative Stent Status on Stone-Free Rates for Ureteroscopic Lithotripsy

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Introduction: Ten to twenty percent of all kidney stones require surgical treatment. Ureteroscopic lithotripsy is indicated for most ureteral stones and many renal stones measuring <2 cm in size. Ureteral stents are often placed to help relieve ureteral obstruction during an acute stone episode. Therefore, a certain number of patients undergoing ureteroscopy will have a stent in place at the time of surgery. **Purpose:** Preoperative stents in patients undergoing ureteroscopy may be beneficial, as the stent passively dilates the ureter and better allows for placement of a ureteral access sheath. We hypothesized that preoperative stent status would be associated with higher stone-free rates in patients with stone burden greater than 5mm, but not in patients with stone burdens less than/equal to 5mm. **Methods:** Records of 436 patients who had undergone ureterosocpy over a 3-year period were retrospectively reviewed. Variables collected were patient's body mass index, location of the stone, stone burden, Hounsfield units of the stone, preoperative stent status, and whether the patient was stone-free post-operatively. Stone-free was defined as a lack of visible stones on KUB or <4mm on a follow-up CT scan. Patients who did not experience post-operative recurrence of symptoms were also considered to be stone-free. Pearson Chi-Square Tests were run to determine whether preoperative stent status was significantly associated with stone-free rate. **Results:** 436 patients were included in the study and were then grouped according to their stone burden: greater than 5mm (group A) or less than/equal to 5mm (group B). In Group A, patients who were stented preoperatively were more frequently considered stone-free (76.6%) than those who were not stented (59.1%) [p = 0.001]. In Group B, patients who were stented preoperatively were also more frequently considered stone free (84.4%) than those who were not stented (59.1%) [p = 0.013]. **Conclusion:** Preoperative stent status is associated with higher stone-free rates for patients undergoing ureteroscopic lithotripsy. Reasons for causality could be explored in a future study.

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Do Amyloid-like Complexes Control Development? An Examination of Amyloids in *Xenopus* Oocytes and Early Embryos

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Successful development of a fertilized egg into a complex multi-cellular organism requires coordinated regulation of both temporal and spatial events. In many animals early development proceeds in the absence of new transcription and must depend upon maternal factors for regulatory signals and responses. Activation of maternal molecules must be regulated to coordinate proper development. For example, information can be inherited as mRNA and translation can be delayed until the protein is needed. Alternatively, inherited proteins may be present but require modification or processing to become active. We present evidence for a previously unappreciated mode of control; *Xenopus* embryos inherit a maternal pool of proteins in amyloid complexes. Using X-34, an amyloid specific fluorescent dye, we have observed amyloid containing structures within sections of developing oocytes. This analysis reveals that the number, size and intensity of amyloid containing particles increase as oocyte maturation progresses. Injection of the non-toxic dye into embryos has allowed us to begin to analyze the dynamic nature of these endogenous non-pathogenic amyloid containing structures throughout development. Our initial studies indicate that these structures exist within the nucleus and cytoplasm, vary with respect to size and intensity of staining and are dynamic with respect to their position within a cell over time. Inheritance of X-34 positive particles from mother to daughter cells was also observed. We have further started studies to examine proteins with putative disaggregase activity to identify their roles in early development. We propose that early embryos use amyloid disaggregases to manage proteins during development.

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Inhibition of Class I Histone Deacetylases at Reperfusion Attenuates Ischemia-Reperfusion Injury and Modifies Mitochondrial Acetylation

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Although rapid reperfusion of ischemic tissue is the treatment of choice for myocardial infarction, much of the resultant damage occurs as a result of reperfusion itself. Previously, we have shown that pretreatment with MS-275, a selective class I histone deacetylase (HDAC) inhibitor, rescues left-ventricular (LV) function and substantially reduces the area of infarcted tissue in isolated rat hearts subjected to ischemia-reperfusion (IR) injury. We tested the hypothesis that MS-275 treatment *at reperfusion* reduces LV tissue damage and improves post-ischemic LV contractile function. Hearts from male Sprague-Dawley rats were isolated and perfused *ex vivo* on a Langendorff perfusion apparatus. A saline-filled balloon was inserted into the left ventricle of the heart to monitor ventricular pressure development throughout the experiment. Hearts were subjected to 30 minutes of ischemia, followed by 60 minutes of reperfusion. MS-275 was administered at doses of 10nM during the entire reperfusion phase, and resultant functional data were compared to untreated hearts. There was no difference in any measure of pre-ischemic contractile function between groups. MS-275 (10nM) administered at reperfusion significantly improved multiple measures of LV function, including dP/dt^{max}, $-dP/dt^{max}$, developed pressure and minimum diastolic pressure. We also observed a 60% reduction in infarct area of 10nM treated hearts compared to control, as measured by 2,3,5-triphenyltetrazolium chloride (TTC) staining. Unexpectedly, mass spectrometry analysis revealed significant changes in acetylation state of multiple mitochondrial enzymes. Administration of MS-275 during the reperfusion phase of IR is sufficient to partially rescue LV function from reperfusion-induced damage. This study emphasizes the importance of exploring class I HDAC inhibitors for protection against ischemia-reperfusion injury. This project was supported by VA Merit award BX002327 to DRM and the South Carolina Clinical & Translational (SCTR) Institute, with an academic home at the Medical University of South Carolina, NIH/NCATS Grant Number TL1 TR000061. Additional support provided by T32 GM008716 and T32 HL07260.

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Withdrawn

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Bilateral Force Transients Evoked by Single-Pulse Microstimulation in the Pontomedullary Reticular Formation

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The purpose of our research is to investigate the role of the reticular formation after injury to the motor cortex such as occurs in stroke. The pontomedullary reticular formation (PMRF) is a group of brainstem nuclei that gives rise to the reticulospinal tract, a descending motor pathway involved in postural stability and motor control of proximal muscles. The PMRF receives input from the motor cortex and projects bilaterally to the spinal cord where it synapses on motor neurons and commissural interneurons, providing a pathway from uninjured motor cortex to the hemiplegic side of the body after stroke. By measuring EMG and force output patterns evoked by microstimulation of reticulospinal neurons, we were able to determine the motor outputs of the PMRF in the primate. Muscle activity was recorded using intramuscular EMG electrodes implanted bilaterally in 24 muscles (12 on each side) of the trunk and arms in two intact *M. fascicularis* monkeys. Force responses were measured by two force-sensitive joysticks, which the subjects were trained to grasp while performing bilateral force control tasks. A tungsten microelectrode was inserted into the PMRF and 2000 30 μA stimuli were applied at 5 Hz to 300 locations. Using stimulus-triggered averaging, we identified significant EMG activity for 139 (56.4%) and significant force responses for 105 (70.0%) of the 150 PMRF stimulation sites included in the analysis. Most stimulation sites produced bilateral muscle activation consistent with facilitation of the ipsilateral flexor muscles and contralateral extensors and suppression of the ipsilateral extensors and contralateral flexors in the upper limbs. Force responses revealed a corresponding pattern of activity, with 65.3% of force responses directed toward the body (medially and posteriorly) in the ipsilateral arm and 78.6% of force responses directed away from the body (laterally and anteriorly) in the contralateral arm. Force responses were characterized by an average amplitude of 12.0 mN and an average onset latency of 15.9 ms. Our results demonstrate the validity of measuring force responses evoked by single-pulse microstimulation in the central nervous system. They show that the "fencing response" pattern of force output (ipsilateral flexion and contralateral extension) commonly observed following a concussion is recapitulated by stimulation in the PMRF. These findings support the hypothesis that motor impairment following stroke, characterized by abnormal synergy of arm flexors and a loss of individualized muscle control, is caused by increased reliance on reticulospinal pathways.

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Beta-Arrestin 2 Biased Signaling is Required for Central Nervous System Osmoregulation

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Beta-arrestins, originally discovered to act as desensitizers for G protein-coupled receptors (GPCRs), are now recognized to be important signal transducers. One recently described transducer function for beta-arrestin is in response to mechanical stretch induced activation of the Angiotensin II type 1 receptor (AT1R), whereby cellular stretch induces a beta-arrestin-biased conformation of the AT1R stimulating ligand-independent ERK signaling. Since regulation of serum osmolality depends on the function of stretch-sensitive osmoreceptors located specific regions of the hypothalamus, we tested the hypothesis that beta-arrestin plays a role in osmoregulation and water homeostasis. Individual mice were placed in metabolic cages and we measured urine osmolality at baseline and after 18 hours of water deprivation in wild-type (WT) (n=11), beta-arrestin1 KO (n=10), and beta-arrestin2 KO (n=10) mice testing their ability to concentrate urine. In response to 18-hour water deprivation, both WT and beta-arrestin1 KO showed a 1.6 fold increase in urine osmolality (2202 mmol/kg to 3675 mmol/kg and 2318 mmol/kg to 3798 mmol/kg respectively, $p<0.001$ post vs. pre WT and beta-arrestin1 KO, two-way ANOVA). In contrast, beta-arrestin2 KO mice under conditions of water deprivation were unable to increase urine osmolality (2753 mmol/kg to 2498.3 mmol/kg, p=ns; urine osmolality after water deprivation between beta-arrestin2 KO vs. either WT or beta-arrestin1 KO $p<0.01$). These data show an inability to concentrate urine in response to osmotic stress in beta-arrestin2 KO mice. To determine whether the defect in urine concentrating ability was central (hypothalamic) versus renal (collecting duct), a set of experiments was performed where a separate cohort of beta-arrestin2 KO mice received the selective vasopressin-2 receptor agonist, 1-desamino-8-d-AVP (DDAVP, 1.0µg/kg of body weight i.p.). Urine samples were collected at 4 hours after DDAVP injection. DDAVP exposure caused a similar 1.4 fold increase in urine osmolality in both WT and beta-arrestin 2 KO mice (2400 mmol/kg to 3468 mmol/kg and 2634 mmol/kg to 3738 mmol/kg respectively, p=ns WT vs. beta-arrestin2), indicating that the defect in the beta-arrestin 2 mice in response to water deprivation is a failure of central release of vasopressin from the brain. Ongoing experiments are currently testing the role of beta-arrestin2 in regulation of thirst and salt appetite. In conclusion, these data suggest a critical role for beta-arrestin signaling in osmoregulatory regions of the brain and may lead to potential new therapeutics strategies for conditions of volume overload such as in congestive heart failure.

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Oxidative Stress Dynamically Regulates snoRNAs in Cardiac Cells

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Oxidative stress is involved in the pathogenesis of cardiovascular diseases, including coronary artery disease, hypertension, and heart failure. While approaches to modulate oxidative stress have been successful in animal models, therapeutic antioxidants have failed in clinical trials. Our goal is to identify novel molecular aspects of oxidative stress response pathways that are suitable for targeted therapeutics. We have previously shown that small nucleolar RNAs (snoRNAs) from the *Rpl13a* locus are critical mediators of ROS production and oxidative stress, and that they can be targeted *in vivo* by therapeutic antisense oligonucleotides. snoRNAs are short non-coding RNAs that localize to the nucleolus, where they typically guide modifications of ribosomal RNA (rRNA). However, the function of *Rpl13a* snoRNAs in oxidative stress responses is genetically separable from their role in rRNA modifications. Moreover, oxidative stress causes rapid nucleo-cytoplasmic transit of the *Rpl13a* snoRNAs. This is particularly interesting because cytoplasmic localization of snoRNAs has not been previously described. The purpose of the current study was to investigate the mechanistic relationship between oxidative stress and subcellular localization of the *Rpl13a* snoRNAs in cardiac cells. We chose doxorubicin (dox) as a clinically-relevant inducer of oxidative stress, and tested it in a model system of cultured H9c2 cardiomyoblasts. Doxorubicin rapidly stimulated the cytoplasmic accumulation of *Rpl13a* snoRNAs. This re-localization was dependent on NADPH oxidase (Nox) activity and the production of superoxide. Using siRNA, we identified a nuclear form of Nox4 (Nox4D) as an essential component of the doxorubicin response. Finally, to better understand the cytoplasmic accumulation of snoRNAs in response to oxidative stress, we performed RNA-seq analysis of small RNAs in the cytoplasm. We found that oxidative stress results in the cytoplasmic accumulation of not only *Rpl13a* snoRNAs, but also most other snoRNAs from the same structural class (box C/D snoRNA). Our results support a model in which doxorubicin localizes to the nucleus and interacts with Nox4D to generate nuclear superoxide, which releases the snoRNAs to the cytoplasm. Furthermore, our RNA-seq data provide evidence for the presence of many different box C/D snoRNAs in the cytoplasm at low levels during homeostatic conditions, and at significantly increased levels following oxidative stress. Our findings suggest that snoRNAs may regulate processes oxidative stress responses through actions in the cytoplasm. Given that snoRNAs can be effectively targeted with antisense therapies, these non-coding RNAs provide novel potential targets in oxidative stress response pathways.

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Biomechanical and Genetic Expression Analysis of Burn-Induced Bone Loss

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Bone loss is a clinically relevant issue for severe burn injury patients. Burn injury can result in a significant loss of skeletal bone mass in both children and adults suffering total body surface area (TBSA) burn injury above 40 percent, leading to an increased risk of osteopenia and osteoporosis as well as morbidity years after the initial insult has resolved. We sought to gather bone-specific biomechanical data and explore genetic markers for bone resorption and formation in a novel rodent burn injury model. Tibial and lumbar vertebral samples were isolated from control, sham-burn, and burn-injury rodents at 3, 7, 14, 21, 28, and 42 days post-injury. Rodents received a 40% total body surface area burn by scald injury. Tibia and lumbar samples were analyzed. Total bone mineral densities (BMD) were assessed by quantitative computer tomography and compressive biomechanical testing was also performed to determine overall bone strength using a Instron biomaterials testing apparatus. Lastly, real-time PCR was performed on RNA samples isolated from the extracted tibias to assess RANK ligand (RANKL) and osteoprotegrin (OPG). Burn-injured rodents demonstrated lower tibial bone mineral density (BMD) in comparison to controls. Vertebral BMD was consistently lower in the burn group at each time point. Biomechanical strength analysis revealed lower bone strengths in the 40% TBSA rodents. Tibia samples from burn rodents demonstrated a significantly lower 4-point bending strength while vertebral samples showed decreased compressive strength. Real-time PCR analysis demonstrated almost a 50% increase in expression of RANKL in burn animals at day 7 and 21 post-injury. Bone mineral density and biomechanical testing of burn-injury rodents has demonstrated skeletal-specific structural alternations resulting in decreased bone density and strength, consistent with the current literature. The observation of increased bone-specific RANKL expression in the burn-injury group suggests that bone resorption may contribute to these observed defects. With a better understanding of the mechanisms of burn-injury bone loss, targeted therapies can be implemented with the intention of avoiding long term bone pathology.

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Fluid Shear Stresses and Viscosity Regulate Osteoclast Activity

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Aseptic loosening of total joint replacements remains the number one cause of failure leading to revision surgery. Aseptic loosening is the resorption of bone in the absence of infection, which leads to a loss of interlock between the implant and the host and subsequent component migration. The biologic response to polyethylene (PE) wear particles is considered the primary cause of aseptic loosening in total knee replacements (TKRs), but our lab recently found extensive bone resorption in short term (<5 years) clinically successful, non-revised, human postmortem retrievals of cemented TKRs *in the absence of identifiable wear of the polyethylene tray*. This observation caused us to examine alternative causes of aseptic loosening, including supraphysiologic fluid shear stresses (FSS), which we found to be present at the interface between PMMA bone cement and trabecular bone. To test our hypothesis that supraphysiologic FSS can lead to bone resorption in the absence of PE debris, we designed and built a novel device capable of exerting specific FSS on cultured cells in a 24-well plate. This adjustable device was used to examine the dose-response effect of fluid shear stress on RAW 264.7 murine osteoclasts, ranging from sub (<0.5Pa) to supra (>2Pa) physiologic levels, including a static, non-loaded control. Supraphysiologic levels of FSS were made possible by increasing the viscosity of the media using 10% neutral dextran (molecular radius 2x10^6). We found that at these levels of dextran, osteoclast resorption was almost entirely abrogated while tartrate resistant acid phosphatase (TRAP) production was greatly increased. We found a clear dose-response effect of dextran/viscosity such that at greater than 3% dextran (5.74cP), there is a linear decrease in pit formation ability with concurrent linear increase in TRAP production as analyzed by percent area fraction. Therefore we tested the effect of FSS on cells using a punctuated dextran regime, such that dextran was only present during loading of FSS (1Hz, 1hr/day). This normalized the pit formation ability of cultured osteoclasts while allowing for supraphysiologic FSS. Using this regime, we found a decrease in osteoclastic activity at physiologic FSS levels, with an increase at sub and supraphysiologic levels as measured by pit formation and TRAP assays. In conclusion, we found that media viscosity and fluid shear stress are capable of mediating osteoclast responses, which implies that osteoclasts responsible for aseptic loosening may *initiated* by such stimuli in vivo, *before* experiencing any biologic response related to PE debris.

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Conserved Transcriptional Coordination of Autophagy and Lifespan by the Kruppel-like Factors

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Traditionally, aging has been understood as a progressive accumulation of cellular damage that eventually leads to organ dysfunction and vulnerability to disease. In the last three decades, pioneering studies in C. elegans and other model organisms suggest that aging is instead an active process driven, at least in part, at the gene regulatory level through the action of key transcription factors, including daf-16/FOXO and pha-4/FoxA. Almost all manipulations which influence lifespan require tight regulation of autophagy, a cytoprotective recycling process that removes dysfunctional proteins and organelles and it has been shown that autophagy deteriorates with age across phylogeny. The Kruppel-like factors (KLFs) are a conserved subfamily of zinc-finger transcription factors which play broad physiological roles, with 18 currently identified in mammals and three putative KLFs in *C. elegans*. Here we show that KLFs are transcriptional regulators of several crucial genes in the autophagy machinery in multiple mammalian tissues through luciferase reporter and ChIP-qPCR assays. We demonstrate that KLFs also regulate autophagy in lower organisms; formation of fluorescent puncta, representative of autophagosomes containing *lgg-1*::GFP (*C. elegans* homologue of mammalian LC3), was significantly increased in *klf-3* overexpressing *C. elegans* worms and mRNA of 12 autophagy related genes are also induced. Most interestingly, overexpression of *klf-3* in *C. elegans* extends lifespan by ~30%, delays the accumulation of age associated pigments and lengthens reproductive span. Furthermore, single knockouts of the three KLFs in *C. elegans* demonstrate minimal lifespan reduction, while double knockout of *klf-1* and *klf-3* dramatically shortens lifespan. RNAi knockdown of *bec-1*, a gene required for autophagy, abolished the extended lifespan of *klf-3* overexpressing worms. These studies identify *klf-3* as a novel transcriptional regulator of longevity in *C. elegans*. Understanding the transcriptional networks governing aging is critical and has implications for aging as well as age-dependent diseases, which currently pose an enormous burden on healthcare systems worldwide.

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Traumatic Brain Injury Augments Host Response to Pneumonia Through Cholinergic and Neurokinin Pathways

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Background: Pneumonia leads the world as the cause of global disability-adjusted life years lost and its mortality has not changed over the last five decades. Our murine model of mild traumatic brain injury (TBI) demonstrated improved bacterial killing and survival in *Pseudomonas aeruginosa* pneumonia. We examined the signaling pathways responsible for improved survival by investigating the contribution of substance P (SP) signaling and the cholinergic anti-inflammatory reflex in augmenting the host response and survival in pneumonia. **Methods**: TBI was induced using a weight-drop model and 48 hours later. *Pseudomonas aeruginosa* (1.25-5x10^7 CFU) was intratracheally instilled into adult female ICR mice. Pharmacological manipulations investigated the contributions of the substance P-neurokinin-1 receptor (NK-1R) and the α7 nicotinic acetylcholine receptor (nAChR) pathways. Receptor antagonists methyllylcaconitine (blocks α7 nAChR) or CJ-12,255 (blocks NK-1R) were dosed prior to TBI and continued every 12 hours until pneumonia. Agonist treatments GTS-21 (a7nAChR) and GR73632 (NK-1R) were given instead of TBI and continued every 12 hours until pneumonia. Bronchoalveolar lavage was performed 4h post-pneumonia (52h) to assess cell counts and bacterial load (CFU). Mortality was followed for 28 days, expressed as Live/Total(n). Cytokine measurements were performed by ELISA. **Results**: In previous studies, TBI mice survived better following *Pseudomonas aeruginosa* infection compared to sham injury mice ($p<0.01$) and recruited more pulmonary neutrophils ($p<0.001$) resulting in better killing of bacteria ($p<0.01$).These beneficial effects are blocked by NK-1R antagonism. NK-1R agonist treatment was unable to replicate the TBI survival advantage ($p<0.001$ TBI 6/18 vs. GR73632, 0/10), but it increased bacterial clearance and pulmonary neutrophil recruitment similar to TBI levels ($p>0.05$, TBI vs GR73632: 2993 vs 5683 CFU/mL and 5.92e6 vs 4.749e6 cells/mL). In contrast, blocking the cholinergic signaling (α7nAChR) abolished the TBI protective effect (6/18 vs TBI+MLA, 0/9), but also increased cell recruitment ($p<0.001$, 6.18e6 vs 14.08e6 cells/mL), effectively releasing the brake provided by the anti-inflammatory reflex. Agonism of the α7 nAChR was able to improve survival (TBI 6/18 vs GTS-21 5/10), despite having similar levels of bacterial burden and cell recruitment as sham ($p<0.05$, 3.95e6 vs 3.22e6 cells/mL). Agonism of α7 nAChR reduced pro-inflammatory BAL TNF-α and plasma IL-6 levels to levels similar to TBI. **Conclusions**: TBI improvement of survival acts through two different pathways. Substance P mediates improved bacterial clearance and cell recruitment, but is insufficient to increase survival. Acetylcholine signaling through the α7nAChR reduces inflammation and appears to mediate improved survival. These findings suggest that combined activation of these pathways result in novel treatments for pneumonia.

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Control of Neuronal Excitability Through Akt, Wee1, and PKC Signaling Pathways

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Kinases play fundamental cellular roles by serving as a nexus of enzymatic cascades governing intracellular protein signaling and genetic programs throughout the entire lifespan of the cell. The pathophysiology of complex brain disorders are marked by changes in neuronal excitability, synaptic plasticity and brain connectivity attributed to kinase signaling, but the molecular mechanisms underlying these cellular events are still poorly understood. There is an urgent need for new methods for rapidly screening kinase pathways linked to brain disorders. Using the bioluminescence-based luciferase complementation assay (LCA), a powerful, versatile toolkit for measuring protein:protein interactions in live cells rapidly, quantitatively, and flexibly, we assayed 13 kinase pathways identified through a high-throughput screening analysis of 400 small molecule inhibitors. Through extensive dose-dependent validations of structurally-diverse kinase inhibitors and hierarchical clustering, we identified the PI3K/Akt pathway, the cell-cycle regulator Wee1 kinase and protein kinase C (PKC) as part of potentially new regulatory networks modulating neuronal excitability. Furthermore, exposure to inhibitors of GSK3 and Wee1 leads to dysregulation of axonal initial segment polarity in native rat hippocampal neurons, as well as correspondingly suppressed intrinsic excitability measured through patch-clamp electrophysiology by a depolarizing shift in the action potential. These studies provide a stepping stone for the discovery of novel kinase pathways linked to brain disorders, and demonstrates the viability of utilizing innovative biochemical approaches towards discovery of brain disorder pathophysiology.

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Differential Enhancement of CD8$^+$ T Cells by Heat-Labile Enterotoxins LT-IIb and LT-IIc from Enterotoxogenic *Escherichia coli* by Intradermal Administration

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Vaccination remains one of the most efficient and cost-effective methods for combating infectious diseases. Many conserved antigen (Ag) targets, however, are poorly immunogenic. Additionally, modern vaccine preparations frequently contain only protein subunits and fail to confer protection against complex pathogens that require both humoral and cellular immunity. These deficits in current vaccine approaches can be overcome by the use of appropriate adjuvants. Heat-labile enterotoxins (HLT) produced by some enterotoxigenic strains of *Escherichia coli* have been characterized as strong adjuvants that elicit robust antibody responses when co-administered with protein antigens in both mucosal and intradermal (ID) routes. Enhancements to the CD8$^+$ T cell compartment by these HLT adjuvants, however, have yet to be evaluated. Utilizing a murine ID immunization model, the adjuvant properties of LT-IIb and LT-IIc, two type II HLTs, were compared with those of LT-I, a prototypical type I HLT. While all three HLT adjuvants enhanced Ag-specific humoral responses to similar levels, LT-IIb and LT-IIc, in contrast to LT-I, induced a more vigorous Ag-specific CD8$^+$ T cell response and proffered faster clearance of *Listeria monocytogenes* in a challenge model. Additionally, LT-IIb and LT-IIc induced distinct differences in the profiles of the Ag-specific CD8$^+$ T cell responses. While LT-IIc stimulated a robust and rapid primary CD8$^+$ T cell response, LT-IIb exhibited slower CD8$^+$ T cell expansion kinetics with better induction of effector-memory cells. Thus, LT-IIb evoked superior long-term protection after immunization in comparison to both LT-IIc and LT-I. Furthermore, LT-IIb and LT-IIc enhanced the total number of dendritic cells (DC) in the draining lymph node (DLN) and expression of costimulatory molecules CD80, CD86, and CD40 on DCs. Finally, in contrast to LT-I, LT-IIb and LT-IIc induced less edema, cellular infiltrates, and general inflammation at the site of ID injection. Thus, LT-IIb and LT-IIc present as attractive comprehensive ID adjuvants with unique characteristics that enhance both humoral and cellular immunity.

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Probing the Dynamics of Central Carbon Metabolism with Isotopic Labels and Comprehensive Chemical Profiling

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Changes in central carbon metabolism (CCM) are important biomarkers of diverse disease states ranging from diabetes, heart failure, neurodegenerative disorders, and cancer. Although the pathways of CCM are well-characterized biochemically, their regulation, especially in the face of changing substrate availability and intracellular compartmentalization of intermediates, remains incompletely understood. Current metabolomic technologies, which attempt to identify and quantify the complete chemical space of a biological system, represent an attractive tool for probing the regulation of CCM, because they are able to describe interacting pathways better than traditional approaches targeted to only a small subset of all the metabolic reactions present within a system. Recent advances by our group have augmented traditional metabolomic profiling techniques based on liquid chromatography/mass-spectrometry (LC/MS) with the use of isotopically labeled substrates in defined combinations to reveal differences in flux through competing pathways. This approach extends current metabolomics platforms to encompass the temporal nature of metabolic flux, while avoiding the complexity of constructing and interpreting the kinetics-based mathematical models found in traditional approaches to flux. Applying our platform to transgenic mouse models harboring a discrete lesion in a pathway that shuttles ketone bodies, we test the hypothesis that ketone body metabolism coordinates carbon flow from glucose and fatty acids through central oxidative and biosynthetic pathways in both mitochondria and cytoplasm. Contrary to previous understandings of CCM, we reveal that fibroblasts and macrophages leverage ketone bodies as key intermediates of glucose and fat metabolism. The uncovering of these previously unappreciated aspects of CCM demonstrates the ability of our metabolomics platform to easily access metabolic dynamics without the need to build complex quantitative models.

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Heme Oxygenase-1 Expression Regulates Mitochondrial Dynamics in Doxorubicin-Induced Heart Failure

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Heme oxygenase-1 (HO-1) is an inducible enzyme that degrades pro-oxidant heme into equimolar quantities of carbon monoxide, biliverdin, and iron. Increased expression of HO-1 confers protection from cardiovascular disease. However, the role of HO-1 expression in doxorubicin (DOX)-induced toxicity, a process that is mediated by oxidative stress and mitochondrial dysfunction, is unknown. We hypothesized that HO-1 expression protects against DOX-induced cardiac injury by preventing mitochondrial toxicity in cardiomyocytes. Wild-type (WT) and humanized HO-1 overexpressing (HBAC) mice were treated with DOX (18 mg/kg of body weight, administered IV over one week as three 6 mg/kg doses) and cardiac function was assessed 14 days after treatment by echocardiography. HO-1 overexpression in HBAC mice prevented DOX-induced dilated cardiomyopathy, which was characterized by systolic dysfunction (ejection fraction 67% vs 51% in WT mice, $P<0.05$, n=5), dilation of the left ventricle (3.22 mm vs 3.55 mm in WT mice, $P<0.05$, n=5) and left ventricular wall thinning (0.89 mm vs 0.61 mm in WT mice, $P<0.05$, n=5). Histological evaluation demonstrated that global and cardiac-specific overexpression of HO-1 prevents cardiomyocyte cytoplasmic vacuolization and loss of myocyte striations in WT mice treated with DOX. DOX also causes significant inflammation in the heart, resulting in an increased proportion of bone marrow-derived ($CD45^+$) cells in the heart of WT mice relative to vehicle treated controls (1.42% vs 0.62%, $P<0.01$, n=5), while HO-1 expression reduced the infiltration of $CD11b^+$ mononuclear phagocytes into the heart following DOX treatment ($P<0.05$, n=5). Transmission electron microscopy (TEM) demonstrated that cardiac-specific overexpression of HO-1 ameliorates DOX-mediated ultrastructural changes to cardiomyocytes by preventing dilation of the sarcoplasmic reticulum, cytoplasmic vacuolization, and myofibrillar disarray at days 14 and 60 after treatment. TEM analysis also demonstrated that cardiac HO-1 overexpression prevents DOX-induced mitochondrial disorganization, which was characterized by striking mitochondrial fragmentation as well as an increased incidence of damaged mitochondria in autophagic vacuoles. Western blot analysis revealed that DOX inhibits the expression of mitofusin (MFN)-1 and -2, which are proteins that regulate mitochondrial quality control by promoting mitochondria elongation through fusion of the outer mitochondrial membrane. HO-1 overexpression restores normal expression of MFN-1 in mice treated with DOX, suggesting a role for HO-1 in protecting the heart from oxidative injury by regulating mitochondrial dynamics.

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Magnetic Resonance Imaging Using High Dynamic Range Acquisition and Algorithm

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T1- and T2-weighted Magnetic Resonance Imaging (MRI) of anatomical regions with a large range of T1 or T2 results in feature loss due to signal saturation or low signal-to-noise ratio (SNR). Adjusting the repetition (TR) and echo times (TE) of a pulse sequence either improves SNR at the expense of more saturation, or mitigates saturation at the expense of SNR. To overcome this limitation, a High Dynamic Range (HDR) processing algorithm from photography was utilized to merge multiple MR images acquired at different TR and TE to generate an image that minimizes feature loss. A method to parameterize HDR processing for MRI data was developed, and High Dynamic Range MRI was validated both in solution phantoms and via in vivo mouse imaging.

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Differential Cycling Method for the Analysis of Phase-Shifts in Circadian Rhythms

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Dysregulated circadian rhythms can detrimentally affect individual health and well-being. Studies have analyzed the role of circadian rhythms on gene expression, comparing different conditions under which rhythmic genes change their cycling characteristics and relating them to organism-wide physiological changes. These characteristics are often separately calculated for each experimental condition, after which they are compared. Unfortunately, experimental limitations on the amount and quality of the data collected often introduce uncertainty into these comparisons. We present a method that compares the experimental conditions to one another directly and accounts for the noise inherent in the measurements. We apply our method to published genome-wide circadian data, demonstrating that our method eliminates incorrectly identified cycling differences, providing clearer biological interpretation of results.

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Yap Reprograms Glutamine Metabolism to Support Hyperproliferative Growth During Liver Development and Tumorigenesis

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Hepatocellular carcinoma is a global health problem with poor prognosis and limited therapeutic options. Recently, the Hippo pathway has emerged as a master regulator of organ size control and tumorigenesis. However, the exact molecular mechanisms and metabolic impact of the pathway in supporting tissue growth and tumor formation are poorly understood. Using a transgenic zebrafish model with liver-specific activation of the transcriptional co-activator Yap, the downstream target of the Hippo pathway, we have shown that Yap promotes embryonic and adult hepatomegaly. These livers show signs of dysplasia but do not form frank tumors. Upon exposure to the chemical carcinogen dimethylbenzanthracene (DMBA), however, transgenic YAP fish demonstrate dramatically accelerated tumor formation. In order to identify genes that may contribute to the substantial hyperproliferative properties of Yap prior to tumor formation, we performed transcriptomic analysis (RNA-seq) in wild type and transgenic Yap livers. Interestingly, expression of genes involved in nitrogen metabolism were significantly altered in transgenic Yap livers. Particularly striking was a greater than 10-fold increase in glutamine synthase, a central regulator of nitrogen metabolism in the liver. Metabolomic analysis of more than 250 polar metabolites in wild type and transgenic livers revealed a corresponding decrease in the abundance of urea cycle intermediates while glutamine was increased in transgenic livers. We conclude that Yap induces metabolic reprogramming in the liver, resulting in decreased ammonia detoxification and increased ammonia assimilation into glutamine prior to tumor formation. Intervention studies with the glutamine synthetase inhibitor methionine sulfoximine established that elevated glutamine synthetase activity contributes to the rapid liver growth observed during Yap-driven hepatomegaly. Finally, studies in cultured human cancer cells identify glutamine synthetase as a Yap target gene, confirming that the glutamine synthetase regulation by Yap is evolutionarily conserved. We conclude that Yap regulates glutamine synthetase expression and reprograms nitrogen metabolism to potentially provide essential components for rapid cell proliferation, which contributes to liver growth during development and tumorigenesis.

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Type 2 cGMP-Dependent Protein Kinase Activates Anti-Neoplastic Signaling in the Colon

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Signaling through cGMP has emerged as a potentially important suppressor of tumorigenesis in the colon but the mechanisms are poorly defined. Studies of guanylyl-cyclase (GCC) knockout mice indicate that cGMP signaling inhibits proliferation in the colon and that AKT may have an important role. Type-2 cGMP-dependent protein kinase (PKG2) is a central effector of cGMP in the colon epithelium and likely mediates the suppressive signaling. An important growth-inhibitory effect of AKT is the inactivation of forkhead transcription factors (FoxO). FoxO proteins are tumor suppressors that regulate growth-inhibitory and pro-apoptotic target gene expression but these proteins have not previously been examined in the intestinal epithelium. The present study sought to determine whether PKG2 can activate FoxO in colon cancer cells and in the colon epithelium. Activation of PKG2 in LS174T colon cancer cells inhibited cell proliferation but did not significantly affect apoptosis. It was found that PKG2 inhibited the activity of AKT but not ERK in these cells. Activation of PKG2 also significantly increased luciferase reporter activity driven by FoxO-responsive elements in LS174T cells. In support of FoxO activation, PKG2 also increased the expression of several FoxO target genes, including catalase, GADD45, and $p27^{kip}$. Treatment of colon explants with 8Br-cGMP also activated FoxO target gene expression at both RNA and protein levels, and this was associated with reduced redox stress. The regulation of FoxO by cGMP *in vivo* most likely requires PKG2, because target gene expression was reduced in the colon mucosa of *Prkg2*$^{-/-}$ mice compared to wild type siblings. Since FoxO has not previously been studied in the colon, we first examined expression using IHC. The data showed that FoxO3a is the most prominent isoform, and was concentrated almost exclusively at the lumenal border. While FoxO1 showed negligible staining, FoxO4 was observed deeper in the crypt. In order to determine whether cGMP could activate FoxO *in vivo*, the PDE-5 inhibitor vardenafil (Levitra™) was used. Mice treated with vardenafil showed a dramatic mobilization of FoxO3a to the nucleus of lumenal epithelial cells. Analysis of the colon mucosa from treated animals showed increased levels of FoxO target genes and reduced redox stress. This was likely to be mediated by FoxO3a since vardenafil did not significantly affect the localization of FoxO1 or FoxO4. Taken together these data define a novel signaling pathway in the colon epithelium, where PKG2 leads to activation of FoxO. The established tumor suppressor role of FoxO proteins highlights the potential therapeutic role for cGMP signaling in colon cancer prevention.

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EZH2 in B Lymphocyte Commitment

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EZH2 is the histone methyltransferase subunit of the Polycomb Repressive Complex 2 that catalyzes the repressive histone modification, H3K27me3. *We hypothesize that EZH2 is required to repress alternative lineages during B cell development.* Loss of EZH2 in hematopoietic cells leads to blocks in B and T cell development. Here we use an *Il7ra*^{cre} mouse model to delete *Ezh2* at the common lymphoid progenitor (CLP) stage to study the role of EZH2 in B lymphocyte commitment. We confirm that *Il7ra*^{cre}*Ezh2*$^{fl/fl}$ mice have a cell-intrinsic block at the pro-B cell stage of development. Although there are normal numbers of pro-B cells in *Il7ra*^{cre}*Ezh2*$^{fl/fl}$ mice, EZH2-deficient pro-B cells are outcompeted by WT cells in competitive chimeras. By qPCR we find that there is modest upregulation of *Gata3* and *Nfil3*, which regulate innate lymphocyte development. Future studies will investigate the developmental plasticity of *Ezh2*-deficient pro-B cells.

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Current Practice and Adherence to the National Comprehensive Cancer Network (NCCN) Guidelines in Endometrial Caner in the National Cancer Database (NCDB)

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Introduction: Endometrial carcinomas (EC) account for approximately 52,000 newly diagnosed cases each year, and most women are cured with the possible combination of surgery, chemotherapy, and radiation (Homesley). In 3 published trials, whole pelvic radiation or vaginal brachytherapy reduced local recurrence, but did not improve survival in early stage carcinomas (Creutzberg, Keys). GOG249, which has completed accrual, may lead to the use of chemotherapy with VG in high-grade stage I tumors (HGS1). All patients with stage 3 EC benefit from adjuvant chemotherapy, regardless of histology (Huh). This is a retrospective study using the National Cancer Database to understand national trends in using surgery, radiation and chemotherapy in EC. **Methods:** We identified women with EC diagnosed between 1998 and 2012 and treated with surgery, chemotherapy and/or radiation using the National Cancer Database. Using Chi-squared tests and multivariate logistic regression, we analyzed pathologic stage of EC by age, grade, histology, facility type, race, payor status, income, location, Charlson score, year of diagnosis and facility location. **Results:** Patients were more likely to have advanced stage disease with increasing age (8% age < 50, 20% age 50-59, 31% age 60-29, 40% age >70). African Americans were at higher risk of being diagnosed with advanced stage disease than patients of white or "other/unknown" race. 40% of all patients were treated in academic centers while 52% were treated in comprehensive community cancer centers. 84% of patients had a full staging surgery, while the remaining patients did not have their ovaries removed, cervix removed, or possibly did not have surgery. In patients with HGS1, rates of brachytherapy increased over time,

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from 7.39% in 1998 to 21.13% in 2012 and the use of chemotherapy increased from 1.57% to 14.13%. There was a significant increase in tri-modality treatment over time in patients with HGS1 (0.36% to 7.77%). There was also an increasing trend of use of surgery and chemotherapy only for HGS1 over time (1.16% to 6.29%). For patients with stage 3, there were 23.17% of patients that only received surgical treatment and did not receive the standard of care. **Conclusion:** Age and African American race increased the risk of being diagnosed with advanced stage EC. The use of adjuvant treatment is changing overtime, likely influenced by published clinical trials. However, there are a significant number of patients that are not being treated per the current guidelines, for which additional analysis will be completed.

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Development of a Protease-Activated Cytolytic Peptide Prodrug for Nanoparticle Therapeutics

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Melittin is a peptide toxin derived from bee venom which has been proposed as a cancer therapy due to its cytolytic activity. Yet, its therapeutic applications have been limited by its nonspecific cytotoxicity and rapid clearance from the circulation. We have previously developed a perfluorocarbon nanoparticle system which extends melittin circulation time and enhances intratumoral accumulation. Here, we present a platform for the design of melittin prodrugs which may be delivered to tumors by perfluorocarbon nanoparticle carriers and locally activated by tumor-specific proteases. Our prodrug consisted of melittin joined to a blocking sequence via a matrix metalloproteinase 9 (MMP-9)-cleavable linker. MMP-9 is a protease produced by a wide variety of cancers and is closely linked with tumor growth and metastasis. While native melittin was substantially hemolytic (HD50: 2.2 μM) and cytotoxic (IC50: 3.0 μM) to B16F10 melanoma cells, the prodrug demonstrated no hemolysis (HD50: > 100 μM) and only slight cytotoxicity (IC50: > 100μM) at concentrations up to 100 μM. MMP-9 cleavage restored the hemolytic activity (HD50: 3.8 μM) and cytotoxicity (IC50: 9.3 μM) of the prodrug. Incubation of the prodrug with perfluorocarbon nanoparticles resulted in stable loading of 10,250 peptides per nanoparticle with no significant change in nanoparticle size. Addition of 20 nM MMP-9 led to activation of 57.8% of nanoparticle-bound prodrug within 3 hours. Furthermore, B16F10 melanoma cell viability was virtually eliminated following incubation with 50 μM free or nanoparticle-bound prodrug in the presence of MMP-9 ($p < 0.001$ compared to control). Nanoparticle accumulation in lung tumors was demonstrated by both fluorescence microscopy and quantitative ^{19}F magnetic resonance spectroscopy in a mouse model of B16F10 lung metastasis. Based on these results, protease-activated melittin prodrugs show great promise for development as highly localized anticancer agents, particularly when used in conjunction with a perfluorocarbon nanoparticle delivery system.

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An ATP-Independent Proteasome Activator Contributes to the Virulence of Mycobacterium Tuberculosis

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Mycobacterium tuberculosis (*Mtb*) is the etiologic agent of the disease tuberculosis, which kills approximately 1.5 million people each year. *Mtb*'s arduous treatment regimen and the recent emergence of increasingly drug-resistant *Mtb* strains have given rise to an urgent need for the development of new anti-tubercular drugs. One promising therapeutic target is the mycobacterial proteasome, a large proteolytic complex that is essential for *Mtb* to cause lethal infections in animals. The only pathway known to target proteins for proteasomal degradation in bacteria is pupylation, which is functionally analogous to eukaryotic ubiquitylation. However, while ubiquitin-independent degradation is known to occur in eukaryotes, no pupylation-independent degradation pathways have been described in bacteria. To identify proteasome cofactors that might contribute to such pathways, we isolated proteins that interacted with proteasomes overproduced in *Mtb* and found a previously uncharacterized protein that bound in high abundance, which we refer to as PafE (proteasome accessory factor E). PafE formed oligomeric rings, bound to the ends of the proteasomal core particle using a conserved carboxyl-terminal motif, and enhanced proteasomal degradation of peptides and an unfolded protein in an ATP-independent manner. These characteristics were reminiscent of the 11S activators, a family of proteasomal cofactors that mediate ubiquitin-independent degradation in eukaryotes but have not been previously described in bacteria. To determine if PafE mediates the degradation of proteins, we identified putative PafE-dependent proteasome substrates in *Mtb* and found that PafE promoted the robust in vitro degradation of one of these substrates, the heat shock repressor HspR. Importantly, an *Mtb pafE* mutant had a general growth defect, was sensitive to heat stress, and was attenuated in a mouse infection model. Collectively, these data demonstrate that PafE mediates a previously unknown protein degradation pathway that contributes to the virulence of one of the world's most devastating pathogens.

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Fluorescein as a Contrast Agent for Confocal Intra-Operative Imaging of Basal Cell Carcinoma

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Background: Among several non-invasive optical imaging modalities that are being developed for the intra-operative imaging and detection of basal cells carcinomas (BCCs), reflectance confocal microscopy (RCM) has shown significant advancement. Similar to other light scattering-based imaging modalities, however, the low specificity of RCM remains a barrier to detect small tumors. Fluorescence confocal microscopy in combination with a contrast agent could improve the accuracy of detecting small tumors. To date, few fluorescence contrast agents are safe for use in vivo: fluorescein, indocyanine green (ICG), and methylene blue (MB). Fluorescein has been used in skin *in vivo* through intradermal injection, and is explored further in this study for intra-operative topical application. We demonstrate the potential to improve intra-operative confocal microscopy imaging of small tumors and potentially guide treatments of BCCs. **Methods**: We conducted a study to determine the optimum conditions (immersion time and concentrations) for maximal efficacy of the fluorescein to enhance contrast of cellular structures. Concentrations of fluorescein of 6.0mM and 0.6mM with soaking times of 5, 15, 30, and 60 seconds were tested. Concentrations were tested in discarded tissue containing BCCs from Mohs surgery. For each combination of concentration and time, 3 specimens were imaged to test repeatability. In total, 24 specimens of BCCs from Mohs surgery were imaged with both reflectance and fluorescence confocal imaging. To digitally colorize mosaics to mimic hematoxylin and eosin staining, acetic acid 35% for 30 seconds was used to enhance nuclei on reflectance confocal imaging. 3 samples were tested for repeatability. The feasibility of using fluorescein for image-guided laser ablation of BCCs, 1 sample was treated with fluorescein and imaged after ablation with a CO2 laser. All mosaics were analyzed by comparison to the corresponding frozen pathology. **Results**: We found that the optimal parameters for fluorescein staining are 0.6mM of fluorescein with time of 60 seconds. Fluorescein alone enhances dense cellular areas while attenuating collagen, providing clearer distinction between tumor and dermis. Acetic acid followed by fluorescein staining darkens adnexal nuclei further improving tumor imaging. Furthermore, the combination of acetic acid and fluorescein may also stain ablated tissue. **Conclusions**: Tissues stained with 0.6mM of fluorescein for 60 seconds without rinsing produced the most vivid images. Acetic acid combined with fluorescein may act as an effective contrast agent to enhance cellular detail in FCM. Future studies will incorporate these parameters *in vivo* and use image processing of RCM and FCM *in vivo* to create digitally colorized confocal images and better detect BCC tumors.

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What is the Positive Predictive Value (PPV) of the Diagnosis "Suspicious for High Grade Urothelial Carcinoma (SHGUC)" in Urinary Tract Cytology (UTCy)?

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Background: In an effort to standardize the practice of UTCy, a new reporting system (The Paris System, TPS) has been proposed, containing new diagnostic categories, including SHGUC. However, the PPV associated with this reporting category of SHUC is currently unknown. Since in our institution SHGUC has been diagnosed for over 10 years, we have reviewed our experience with SHGUC to determine the PPV associated with this diagnosis. **Design:** Cases diagnosed as SHGUC and positive for high grade urothelial carcinoma (PHGUC) from 01/01/2003 to 12/31/13 on urinary cytology which had any surgical biopsy, cytology performed within 6 months following the index test were included in the study. During the study period our institution processed 17,770 urine cytologies with 2.75% diagnosed as PHGUC and 1.00% diagnosed as SHGUC. **Results:** We identified 665 cases (178 SHGUC, 487 PHGUC), corresponding to 389 unique patients, 76% men with a mean age of 73.4 and 24% women with a mean age of 74.3. 149 had follow-up via histology only, 52 via cytology only, and 464 had both surgical and cytological follow-up. Cases were considered positive for malignancy if at least one follow-up method resulted in a positive diagnosis (Table 1). **Conclusion:** The PPV of PHGUC (87.1%) was much higher than that of SHGUC (55.6%) ($p<0.0001$). The PPV of PHGUC did not vary according to the indication for urinary tract cytology ($p=1$); while the PPV of SHGUC appeared higher in patients followed for UC, but the difference was not statistically significant ($p=0.19$).

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Biophysical Basis of Receptor Recognition by Chaperone-Usher Pili in *E. coli*

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Extracellular fibers called chaperone-usher pathway (CUP) pili are critical virulence factors in Gram-negative bacteria that mediate adhesion to host and environmental surfaces. Uropathogenic *E. coli* (UPEC) use the type 1 pilus adhesin FimH to bind mannosylated receptors on bladder epithelium to establish urinary tract infection. Recent studies indicate that positively selected residues, which lie far away from the mannose binding pocket, impact the functions of FimH in UPEC pathogenesis, but the mechanism by which this occurs remains poorly understood. Moreover, the molecular basis for the function of divergent FimH homologs remains unknown. Here, we investigate the ligand specificity, conformation, and evolution of FimH-like adhesins to understand the structural, dynamic, and allosteric parameters that govern interactions at the host-pathogen interface. The effects of positively selected residues on the conformation of FimH while bound to periplasmic chaperone FimC or pilus adaptor peptide $FimG_{Nte}$ were probed by small-angle

X-ray scattering (SAXS). As expected, all tested FimH variants are held in an elongated state when bound to FimC. However, the same variants within the $FimG_{Nte}H$ complex vary drastically in conformation to one another, indicating that FimH sequence modulates FimH conformation while bound to the pilus adaptor. Measures of protein size and shape by SAXS together with protein thermal stability indicate that, on average, A27V/V163A is "less compact" and A62S is "more compact" compared to WT FimH in a tip-like setting. In support of a conformational selection model, variants that have a higher tendency to adopt the elongated state have a higher affinity for mannose and higher propensity to form biofilms. Despite their sequence divergence, FmlD and SfaH lectin domains adopt the same beta sandwich fold as FimH but differ in their ligand specificity, as determined by X-ray crystallography, glycan arrays, and biochemical assays of carbohydrate and glycoproteins. Further, ancestral adhesin reconstruction by maximum-likelihood phylogenetics and chimeric adhesin engineering has begun to unveil the structural basis of ligand diversification in adhesin evolution. This work has identified binding loop 3 as the determinant of ligand specificity in FimH-like adhesins and has revealed the necessity for residues within binding loops that do not directly interact with ligands in ligand binding, likely through their influence on loop backbone positioning. These studies promise to reveal fundamental principles of bacterial adhesion, which will provide opportunities for the design of innovative anti-adhesive therapeutics to combat multi-drug resistant Gram-negative bacterial infections.

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Electronic Expression of IK1 Optimizes Action Potentials in Human Induced Pluripotent Stem Cell Derived Cardiomyocytes

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Introduction: Cardiac myocytes derived from human induced pluripotent stem cells (hiPSC-CMs) are a useful and renewable human myocyte model. Despite their tremendous promise, these cells have considerable unexplored potential limitations when applied to their quantitative action potential (AP) analysis. HiPSC-CMs have deficient expression of inward rectifying potassium channel (IK1) causing a depolarized membrane potential and contributing to spontaneous APs, inconsistent AP properties, and misidentification of cellular phenotype. **Hypothesis:** We tested the hypothesis that electronic expression of IK1 in hiPS-CMs can restore mature physiological APs. **Methods:** Commercially prepared cells (iCells) from Cellular Dynamics (Madison, WI) and scale production cells (hIPS-CMs) were compared with human adult ventricular myocytes (VM) isolated from biopsy samples. APs where recorded from single myocytes using the single cell ruptured patch technique with a dynamic clamp expression of IK1 current. **Results:** At -120 mV, a large IK1 is observed in VM; IK1 currents are much smaller in iCells and hIPS-CMs. Electronic expression of IK1 eliminates spontaneous behavior and restores normal physiological parameters. A normal resting diastolic potential was reestablished with application of IK1. Spontaneous early and delayed after depolarizations where reduced and beat-to-beat variability was diminished. Despite a detectable transient outward potassium current in hiPSC-CMs, "spike and dome" morphology is generally absent in spontaneously active cells; addition of electronic IK1 restored this morphology and the relationship between maximum upstroke velocity and sodium current density. The stabilized membrane potential allowed systematic measurement of dynamic parameters. The rate dependence of the AP duration was measured in at different pacing rates in electronic IK1 expressing hiPSC-CMs and showed a classical monotonic restitution curve, with AP increasing with increased cycle length. Also, electronic expression of IK1 allowed for improved discrimination of atrial and ventricular cells based on AP morphology. **Conclusions:** Our study demonstrates that electronic expression of IK1 is a simple and robust method to significantly improve the physiological behavior of the electrical profile of hiPS-CMs. The restoration of a normal cardiac resting potential enables the full recovery of the ion channels, allowing them to have a more normal physiological role, which is important for studying disorders such as long QT, Brugada, and J-wave syndromes. Electronic expression permits the identification of various types of repolarization dysfunction and can improve hiPSC-CMs drug safety and efficacy assays.

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Characterizing the Phosphorylation Dependent Binding Interactions of Cardiac Myosin Binding Protein C to Modify Cardiac Contractility

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Cardiac myosin binding protein-C (cMyBP-C) is a sarcomeric accessory protein that binds to regions of the myosin head to depress force generation. Phosphorylation of cMyBP-C by PKA and CAMKII increases cardiac contractility following adrenergic stimulation by attenuating the interactions of cMyBP-C with myosin and actin. Failure of this mechanism due to decreased adrenergic signaling in heart failure most likely contributes to reduced contractility. In addition, mutations in cMyBP-C may disrupt the interactions of cMyBP-C with myosin, thereby contributing to hypercontractility in hypertrophic cardiomyopathy. cMyBP-C contains three phosphorylatable serines at the N-terminus that exist in a range of phosphorylation states in the sarcomere. However, the functional effects of these partial phosphorylations are not known. To test the hypothesis that the three phosphoserines accelerate contraction kinetics in a graded manner, we are generating mutated mouse engineered cardiac tissue expressing systematic combinations of serines, aspartes, or alanines to mimic WT, phosphorylation, and dephosphorylation respectively. We will quantify the relative contributions of the three phosphoserines to accelerate contraction kinetics by recording twitch force and $[Ca^{2+}]_{in}$ kinetics. Furthermore, to determine whether the phosphorylation state of cMyBP-C influences its binding to myosin, actin, or both, the phosphoserines of bacterially expressed N-terminal fragments of cMyBP-C will be mutated in similar systematic combinations. The effect of these mutations on myosin and actin binding will be characterized with co-immunoprecipitation and surface plasmon resonance. Understanding the binding partners of cMyBP-C after post translational modification will provide therapeutic targets for hypocontraction in heart failure and hypercontractility in hypertrophic cardiomyopathy.

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Nociceptive Sensory Fibers Drive IL-23 from CD301b+ Dermal DC and Provide Protection from Cutaneous C. *albicans* Infection

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The skin is a highly immunologically active barrier site that provides protection against invading pathogens. It is also a highly innervated sensory interface that can directly sense pathogens via nociceptive pathways resulting in pain. Innate resistance to *C. albicans* in mucosal tissues depends on early production of IL-17A by tissue resident cells. In the skin, we found that CD4+ TCRαβ T cells andTCRγδ T cells but not innate lymphoid cells rapidly produce IL-17A in response to C. albicans infection. Efficient clearance required Vγ4 TCRγδ T cells but not TCRαβ cells. In addition, production of IL-17A by TCR γδ T cells as well as their proliferation depended on IL-23 derived from CD11c+ DC. Using mice lacking individual skin-resident DC subsets, we found that induction of IL-17A from TCRγδ T cells and resistance to C. albicans required IL-23 from CD301b+ dermal DC. Moreover, RTX inhibition of nociception, cutaneous denervation, and inhibition of the neuropeptide CGRP resulted in a reduction in the number of IL-17A+ TCR γδ T cells and increased susceptibility to C. albicans infection. These data define a model in which nociceptive pathways in the skin drive production of IL-23 by CD301b+ dermal DC resulting in IL-17A from TCR γδ T cells and resistance to cutaneous candidiasis. The ability of nociceptive fibers to interact with skin resident DC and augment local resistance to a pathogen represents a novel pathway that could be targeted to augment antimicrobial therapy in the skin.

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Combinatorial Cell Microarrays for Analyzing Extracellular Matrix Regulation of Tumor Cell Drug Response

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Carcinoma progression and metastasis are directed by interactions between epithelial tumor cells and components of the tumor cell microenvironment. In particular, cell-extracellular matrix (ECM) interactions have been demonstrated to influence tumor growth, metastatic potential, and sensitivity or resistance to therapy. In small cell lung cancer, for instance, adhesion to collagen IV, fibronectin, and laminin is known to protect tumor cells from drug-induced cell cycle arrest and apoptosis through an integrin-mediated mechanism. Similarly, adhesion to fibronectin in the presence of galectin-3, galectin-8, and laminin has been associated with metastatic lung adenocarcinoma. However, the complexity of ECM composition within the *in vivo* tumor microenvironment has limited the understanding of the underlying mechanisms. Here, we have developed a high-throughput cell microarray-based approach for investigating the impact of defined combinations of ECM proteins on tumor cell phenotype, function, and drug response. Briefly, lung adenocarcinoma (A549) cells were seeded onto a polyacrylamide hydrogel substrate spotted with ECM proteins, treated with drugs for 48 hours, and stained by immunofluoresence for markers of apoptosis, proliferation, and cell phenotype. Using this approach, we quantitatively evaluated the effects of 54 different ECM environments comprising all 2-factor combinations of 10 ECM proteins in response to a panel of drugs including an alkylating agent (cisplatin) and 6 receptor tyrosine kinase inhibitors (cabozantinib, gefitinib, nilotinib, vandetanib, and sunitinib). We further examined if intrinsic gene expression alterations interact with ECM context to define drug response. ASCL1, an important regulatory transcription factor in pulmonary neuroendocrine cell development, has previously been associated with overexpression of the oncogene RET in a subset of lung adenocarcinomas with aggressive behavior and poor patient outcome. We directly compared the responses of wild-type A549 cells and A549 cells expressing ASCL1 and identified cell type-specific drug effects within distinct ECM environments. These studies illustrate the capability to systematically deconstruct the combinatorial role of ECM in tumor cell function and drug response through the application of a high-throughput analysis platform. Continuing work utilizing this approach aims to further define the mechanisms by which interactions with ECM drive lung carcinogenesis and resistance to drugs while integrating cell types relevant to pulmonary neuroendocrine tumors.

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Minicircle Gene Delivery and ACE2 Amplification in Diabetic Mice

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Background: It is generally accepted that in diabetic kidney disease there is overactivity of the renin angiotensin system (RAS). Angiotensin-converting enzyme 2 (ACE2) is a monocarboxypeptidase that hydrolyzes angiotensin (Ang) II to Ang-(1-7) and therefore could play an important role in down-regulating the RAS overactivity. Sustained amplification of ACE2 activity *in vivo* has required the development of transgenic mice or the use of viral vectors. Mini-circle is a new gene delivery technology which is resistant to gene silencing, and therefore represents an attractive platform for gene replacement strategies *in vivo*. **Methods:** Here we used minicircle delivery for chronic ACE2 amplification *in vivo*. The cDNA of soluble mouse ACE2 was cloned into a circular expression cassette and the resulting ACE2 minicircle (MC) was injected to *FVB* mice in which STZ diabetes was induced. **Results:** At 3-7d after MC administration, serum ACE2 activity in mice that received 10ug ACE2MC (n=9) was over 100-fold higher than in controls (n=9) (138±48 vs. 0.7±0.2 RFU/uL/hr) and with a larger dose (30ug) (n=8) increased even further (480±153 RFU/uL/hr). The marked increase in serum ACE2 activity was sustained for several months but did not result in a detectable increase in either kidney ACE2 activity or immunostaining. We reasoned that ACE2MC could be used to examine if chronic amplification of circulating ACE2 affects the development of glomerular lesions in diabetic mice. Control or ACE2MC(10-30ug)-treated mice were either injected with vehicle

or STZ. Urinary ACR measured over the entire 20 wks after the STZ injection was significantly increased in STZ (n=16) and in STZ-MCACE2 mice (n=14) as compared to vehicle controls (n=9). Similarly, a number of patho-physiological indices, including glomerular mesangial score, glomerular cellularity and total glomerular area, were all increased to a similar extent in STZ and STZ/MC mice, as compared to vehicle controls. Consistent with glomerular hypertrophy seen in both STZ-treated groups, the GFR in was significantly increased in STZ (10.8±0.5 **uL/min/g**) and STZ-ACE2MC (10.3±0.5 **uL/min/g**) as compared to vehicle mice (3.6±0.7 **uL/min/g**). **Conclusions**: Mini-circle delivery of ACE2 results in a dose-dependent and sustained long-term increase in serum but not kidney ACE2 activity. A profound augmentation of ACE2 activity confined to circulation is not sufficient to prevent glomerular pathology and hyperfiltration in diabetic mice. Strategies to achieve kidney over-expression of ACE2 are needed to examine its postulated beneficial effect in diabetic kidney disease.

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Structure-Specific Endonuclease XPF-ERCC1 Plays a Critical Role in DNA Interstrand Crosslink Repair that is Compromised in Patients with Fanconi Anemia

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DNA repair is a critical process to prevent genomic instability, a hallmark of cancer. DNA interstrand crosslinks (ICLs) may occur spontaneously via endogenous cellular components or exogenous DNA damaging agents such as the platinum drugs. Because DNA replication is blocked as a result of crosslinks, genomic integrity may be compromised and cell death may occur if replication fork stalling is not resolved. The structure-specific endonuclease XPF-ERCC1 has been reported to be a crucial component for this kind of lesion repair. Mutations in XPF have also been described that give rise to Fanconi Anemia, a childhood disorder characterized by developmental problems, bone marrow dysfunction and a high predisposition to early development of cancer. Using the yeast homolog to XPF-ERCC1, Rad1-Rad10, we focus on the functions of XPF-ERCC1 that are specific for ICL repair and separate the distinct nuclease activities in yeast. We show that this complex is directly involved in 3' flap removal during ICL repair, and that this activity is redundant with another structure-specific endonuclease, Mus81-Mms4, in repairing interstrand crosslinks. In addition, we show that mutations in Rad1 which mimic XPF mutations in patients with Fanconi Anemia are sensitive to ICL damage. Our results indicate that XPF-ERCC1 has specific roles throughout ICL repair and that this endonuclease employs separate nuclease functions to resolve DNA interstrand crosslink lesions in both yeast and mammals. Furthermore, we propose that deficiency in 3' flap removal leads to Fanconi Anemia, thus emphasizing its critical role in suppressing a cancer predisposition.

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Enhancing Dendritic Cell Immunotherapy Through IL-2/mAb Complexes for Control of Established Tumors

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The widespread implementation of vaccines has been one of the most successful medical interventions in public health. With the growing incidence of complex chronic diseases, there is considerable interest in designing therapeutic vaccines or immunotherapies to stimulate natural host defenses against common illnesses such as malignancy. Recently approved dendritic cell (DC) immunization, high-dose cytokine therapy, and checkpoint blockade antibodies offer potential treatment options, but at the risk of poor efficacy, severe toxicity, or indefinite outcomes, respectively. Here, we describe a short-term DC immunization coupled with stabilized IL-2/mAb complex infusion to robustly stimulate both NK cells and tumor antigen-specific effector CD8 T cells. This immunization strategy immediately enhances the number and function of NK cells by ~10-fold and effector CD8 T cells by ~60-fold over DC immunization alone. Additionally, DC priming followed by IL-2/mAb complex treatment selectively upregulates expression of the costimulatory molecules 4-1BB and GITR, and sustains high granzyme B expression on both NK and CD8 T cells. Furthermore, antigen sensitivity for cytokine production and the per-cell killing capacity of effector CD8 T cells is enhanced following DC+IL-2/mAb complex treatment. Importantly, DC prime + IL-2/mAb did not induce expression of inhibitory receptors, and resulted in CTL:TReg ratios >10-fold. This immunotherapy strategy enhanced both endogenous NK cell and tumor antigen-specific CD8 T cell immunity to provide a marked reduction in tumor burden in multiple models of pre-existing malignancy. Specifically, size and number of lung tumor nodules from a transplantable B16 melanoma model were dramatically reduced following DC+IL-2/mAb immunization. Additionally, only DC+IL-2/mAb treatment completely prevented metastasis in a 4T1 breast cancer tumor model in BALB/c mice. These data reflect the broad efficacy of DC+IL-2/mAb immunization for a variety of cancers in genetically diverse backgrounds. Through depletion of CD8 T cells and NK cells, we conclude that amplification of both the innate and adaptive arm of the immune system is necessary for efficient tumor control in this immunotherapy approach. Notably, IL-2/mAb complex treatment of aCD3-stimulated human CD8 T cells resulted in higher number and granzyme B production, supporting the translational potential of this immunization strategy for CD8 T cell-mediated immunotherapy of human malignancy.

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Blocking Viral Access to Heparan Sulfate Inhibits HMPV Infection in Human Lung Cells

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Human metapneumovirus (HMPV) is a recently discovered paramyxovirus that infects nearly 100% of the world population. This enveloped RNA virus causes severe respiratory disease in infants, the elderly, and immunocompromised patients worldwide. In fact, HMPV is estimated to be the second most common cause of pediatric lower respiratory illness. Despite its clinical significance, there is no antiviral treatment or vaccine available. Entry of paramyxoviruses into host cells typically requires the coordinated activity of the attachment glycoprotein, G, which interacts with a cell receptor, and the fusion glycoprotein, F, which promotes subsequent fusion of viral and cellular membranes. Interestingly, unlike other paramyxoviruses, recombinant HMPV without G is replication competent in cell culture and in multiple animal models, suggesting a double function of HMPV F. Our group previously showed that HMPV F mediates binding to target cells, and this interaction requires the presence of the glycosaminoglycan heparan sulfate (HS), a repeating sulfated disaccharide sugar made up of iduronate-2-sulfate and N-sulfo-D-glucosamine-6-sulfate. HS is found on heparan sulfate proteoglycans (HSPGS), which are cell surface proteins that serve as attachment factors and mediate signaling by ligand binding and interaction with proteins influencing major cell processes. HSPGs have been implicated in virus-cell interactions for other enveloped viruses including herpes simplex viruses (HSV), human immunodeficiency virus (HIV), and respiratory syncytial virus (RSV). To tease apart this interaction, we tested compounds known to either occlude HS at the cell surface or interact with potential HS-binding domains on viral proteins by mimicking HS. Variably sulfated derivatives of *E. coli* K5 polysaccharide interact with HS-binding proteins and have reported antiviral activity against human papillomavirus (HPV)-16, HIV, and HSV-1, without anti-coagulant activity *in vivo*. Peptide dendrimer SB105-A10 interacts with HS chains on the cell surface and has reported antiviral activity against RSV and HIV. We found the pretreatment of virus with only the highly sulfated K5 polysaccharides inhibited HMPV infection in multiple human lung cell lines in a dose-dependent manner, suggesting negative charges resulting from sulfation of HS are critical for interaction with the HMPV F protein. Peptide dendrimer SB105-A10 inhibited HMPV infection in a dose-dependent manner as well, suggesting occlusion of HS at the target cell surface is sufficient to prevent viral binding. These results were also seen in a 3D model of the human lung using Human Airway Epithelium tissues, suggesting these interactions take place during HMPV infection in a physiologically relevant model that recapitulates the complexity of the human airway.

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Inhibition of Ezh2 Simultaneously Decreases Pro-Inflammatory and Enhances Anti-Inflammatory Microglial Phenotypes

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The aging central nervous system (CNS) is characterized by a chronic baseline elevation in inflammation which contributes to the pathogenicity of many neurological diseases. Understanding the source of this inflammation is critical as manipulations may allow for the reversal of these age-related changes, thereby reducing the risk of CNS disease. This has led to a focus on the role of microglia, the resident innate immune cell of the brain, in CNS inflammation. Microglia possess the ability to adapt to the environment by altering their phenotype between a resting (M0) state to either an activated pro-inflammatory (M1) or anti-inflammatory (M2) phenotype. In the setting of aging, microglia have a more predominant M1 phenotype at baseline. However, the molecular mechanisms underlying these changes with age remain largely unknown. Recent work has shown that Jumonji Domain Containing 3 (Jmjd3), a histone demethylase, is essential for M2 polarization. By demethylating H3K27me3 to H3K27me1, Jmjd3 alters the epigenetic landscape of target promoters from a transcriptionally repressed state to a transcriptionally active state, thereby allowing for gene expression to occur. In contrast, Enhancer of Zeste Homologue 2 (Ezh2) functions in an opposite manner by selectively writing the H3K27me3 mark. We hypothesized that inhibition of Ezh2 may tilt the balance between Jmjd3 and Ezh2 respective activities in favor of higher Jmjd3 activity, thereby enhancing polarization toward an M2 phenotype. Mixed glial cultures were isolated from P0.5-P2 C57Bl/6 mice and cultured for 10-14 days before microglia were isolated. Primary microglia were treated with GSK343 (an inhibitor of Ezh2) for 1hr before being stimulated with LPS+IFN-gamma or IL-4. After 4hr or 24hr RNA was isolated for qRT-PCR analysis. In our model of primary microglia, pre-treatment with GSK343 significantly diminished LPS+IFN-gamma-induced *IL6* and *IL1B* expression at both 4hr and 24hr ($p<0.05$). Additionally, Ezh2 inhibition rescued the expression of M2-associated genes *ARG1*, *CD206*, and *IRF4* at 24hr ($p<0.05$) which were otherwise significantly down-regulated in the presence of LPS+IFN-gamma. Inhibition of Ezh2 also resulted in an augmented IL-4-induced upregulation of *ARG1*, *CD206*, and *IRF4* after 4hr ($p<0.05$). Our data implies that there is epigenetic regulation of M1/M2 microglia polarization and that Ezh2 has a role in this regulation. Pre-treatment with an inhibitor of Ezh2 limits LPS+IFN-gamma-induced microglial M1 polarization and restores M2-associated gene expression to baseline. This suggests that altering the balance between Ezh2 and Jmjd3 allows for reprograming of both M1- and M2-associated genes.

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Tfap2a, Irf6 and *Grhl3* Interact to Regulate Ectoderm Development

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IRF6, TFAP2A and GRHL3 encode transcription factors that are required for orofacial development in humans and mice. We showed that rs642961 is highly associated with orofacial clefting and perturbs a TFAP2A binding site in an enhancer element (MCS9.7) that recapitulates Irf6 expression. In keratinocyte culture, TFAP2A regulates IRF6 expression via MCS9.7. We also showed that Irf6 regulates Grhl3 expression in zebrafish and that mutations in IRF6 and GRHL3 cause nearly identical human phenotypes. These observations suggest that TFAP2A, IRF6 and GRHL3 form a network in craniofacial morphogenesis. However, mice that lack either Tfap2a or Grhl3 also have neural tube defects. These neurulation defects are located rostrally (exencephaly) and caudally (curly tail) in both mutant mice. In addition, Tfap2a knockout embryos have an exceptional ventral wall defect involving both the thoracic and abdominal cavities. Therapeutically, neurulation defects in Grhl3 knockout embryos are rescued by inositol supplementation, but not folate. In this study, we discover a critical role for Irf6 in neural tube and ventral wall morphogenesis by characterizing an allelic series, including both loss- and gain-of-function alleles. We find that reducing Irf6 expression leads to a curly tail and reductions in both Tfap2a and Grhl3 expression. Remarkably, reducing Irf6 expression in Tfap2a haploinsufficient embryos rescues exencephaly. Irf6 over-expression leads to a ventral wall defect that is highly analogous to Tfap2a knockout embryos. Molecularly, we find that Irf6 is expressed in neural ectoderm and early migrating neural crest cells and that MCS9.7 is active in these cells. Strikingly, we show that Tfap2a regulates MCS9.7 in multiple tissues, including tail and skin. Consistently, we find that Tfap2a and Grhl3 interact genetically in rostral and caudal neurulation and ventral wall development in a dose-dependent manner. Therefore, Irf6 homeostasis is required for at least three ectoderm derived tissues via positive and negative regulation of Tfap2a and Grhl3. Lastly, we sequence IRF6 in 96 individuals with spina bifida and find a rare variant previously shown to be disease causing in orofacial clefting. We further genotype common SNPs in 2,500 individuals with spina bifida to cover 28% of the genetic variation at the IRF6 locus but do not detect a common association. With roles in neural tube, ventral wall and craniofacial morphogenesis, Tfap2a-Irf6-Grhl3 appears to be a key transcriptional gene regulatory network in ectoderm development. Considering that Grhl3 is a distal node in this pathway, inositol supplementation and inhibition may provide an environmental lever to alter multiple developmental programs of the ectoderm based on genetic risk.

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Sensory Deprivation Following Cortical Focal Ischemia Facilitates Remapping And Accelerates Behavioral Recovery

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Introduction: Recovery after focal cortical stroke, often unpredictable and incomplete, is associated with functional remapping to cortical regions adjacent to the lesion. We sought to determine if recovery could be accelerated by enhancing adjacent cortical plasticity using focal sensory deprivation; and if this remapping was dependent on mechanisms involved in synaptic plasticity. **Methods:** Three cohorts of mice were subjected to focal photothrombosis of the right forepaw somatosensory (S1fp) cortex: 1) C57bl6 mice (control, n=11); 2) C57bl6 mice subjected to chronic whisker trimming (sensory deprivation, n=9); and 3) C57bl6 mice with Arc gene deletion (Arc, n=7), a gene critical for synaptic plasticity. S1fp remapping was assessed by electrical forepaw stimulation with optical intrinsic signal (OIS) imaging through the intact skull prior to, and 1, 4, and 8 weeks following photothrombosis. Somatosensory recovery was measured using the cylinder rearing test at 1, 3, 5, and 7 weeks after photothrombosis. ANOVA with repeated measures and Newman–Keuls' multiple comparisons was used to compare each timepoint to baseline. **Results:** Photothrombosis reproducibly infarcted S1fp in all cohorts, resulting in absent S1fp activation maps and reduced right forepaw use (30% more left paw use than right $P \leq 0.001$ in all groups) at wk 1 post-photothrombosis. In control mice, S1fp remapping was first observed at wk 8 in the motor area, and symmetric forepaw use improved along a similar time-course (26% asymmetry at wk 3, $P \leq 0.01$; 17% asymmetry at wk 5, $P \leq 0.05$; 7% at wk 7, P=n.s. compared to baseline). Sensory deprivation induced earlier remapping into the barrel cortex (wk 4), and behavioral recovery (symmetric limb use) also occurred by wk 3 (9% asymmetry, P= n.s.). $Arc^{-/-}$ mice showed no S1fp remapping and limb asymmetry persisted for the entire recovery time course (31% at wk 3, 29% at wk 5, and 27% at wk 7, $P \leq 0.01$). **Conclusion:** Functional remapping following cortical stroke can be redirected to targeted regions by focal sensory deprivation, resulting in accelerated remapping and recovery. These recovery processes are dependent on Arc, which plays an important role in synaptic plasticity.

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Negative Impact of Myeloid-Derived Suppressor Cells on CD8 Effector T Cell Trafficking Within the Tumor Microenvironment

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The success of T cell-based immunotherapy and, unexpectedly, thermal therapy, standard chemotherapy and radiation hinges on cytotoxic T cells gaining access to tumor targets. These observations have prompted interest in strategies to improve T cell trafficking to tumors although the mechanisms that positively or negatively regulate extravasation at tumor vascular checkpoints are poorly understood. Here, we report that the ability of tumor vessels to respond to systemic thermal therapy and other IL-6-dependent preconditioning regimens that boost CD8 effector T cell homing is temporally and inversely related to the expansion of myeloid-derived suppressor cells (MDSC) within the tumor microenvironment. Using real-time intravital imaging and immunofluorescence histology, IL-6-dependent therapies were shown to convert vessels from T cell-low to -high recruitment sites in murine tumors with minimal MDSC infiltration (i.e., CT26 colorectal, B16 melanoma, EMT6 mammary tumors). This conversion requires induction of the prototypical trafficking molecule ICAM-1 on tumor vessels. Conversely, mammary (4T1, AT-3 and PyMT-MMTV) and pancreatic (Pan02) tumors with high MDSC burdens were refractory to IL-6 therapies, but became responsive after acute MDSC depletion. To further investigate contributions of MDSC to poor CD8 effector T trafficking, IL-6-responsive tumors were admixed with syngeneic $CD11b^{+}Gr\text{-}1^{+}$ MDSC isolated from spleens of tumor-bearing mice at a ratio of 2:1, thus mimicking the high MDSC burden detected in IL-6-refractive tumors. Sustained elevation of MDSC in admixed tumors resulted in failure to support increased T cell trafficking in response to IL-6–dependent therapy. Taken together, these findings identify a novel role of MDSC in subverting antitumor immunity by limiting T cell trafficking at tumor vascular loci. Supported by NIH (R01CA79765, R01AI082039, 2T32CA085183) and Mark Diamond Research Fund.

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Intrinsic Spectroscopic Biomarkers for Evaluation of Chemotherapy Response in Breast Cancer

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Spectroscopic methods using near-infrared light provide contrast based on quantitative functional changes in tissue. Near-infrared optical techniques have shown to be sensitive to changes in breast physiology and pathology by revealing quantification of total hemoglobin (oxyhemoglobin and deoxyhemoglobin), oxygen saturation, bulk lipid, and water content. Breast tumors display changes in these parameters from chemotherapy, however they are not particular "signatures" of cancer. There is interest in non-invasive evaluation of breast changes during neoadjuvant chemotherapy for assessment of response to chemotherapy and determination of the next step. The Double Differential spectroscopic method demonstrates intrinsic spectroscopic biomarkers which are specific to malignant tumors. Here we investigate the role of intrinsic spectroscopic biomarkers for monitoring of neoadjuvant chemotherapy. Diffuse Optical Spectroscopy instrumentation was used to recover non-invasively the absorption and scattering spectra from 650-1000 nm in the breast tissues of patients. The subjects were women, both pre and post-menopausal in the age range of 30-60 years. The breast cancer location and pathology was known a priori through imaging and biopsy. Measurements of the optical parameters including hemoglobin, lipid and water were obtained sequentially before and after the administration of chemotherapy. The Double Differential method was applied to analyze the complete absorption spectra of breast tissues. The Double Differential method involves only spectral differences between normal and diseased tissue, and analyzes the residuals of the fit to the basis spectra and normal tissue. Tumor-specific spectral signatures were present in cancerous lesions, and not within normal breast tissues of the same patient. Mapping of the spectral signature demonstrated spatial heterogeneity of the tumor. The spectral changes were quantified by characterizing the spectral features into the Specific Tumor Component index. Spectral changes were identified which could be tracked through the course of chemotherapy. Optical spectroscopic methods utilize a non-invasive, non-radiation approach to demonstrate functional characterization of tissue. This provides a quantitative method for monitoring of response to neoadjuvant chemotherapy. Furthermore Diffuse Optical Spectroscopic Imaging provides a functional complement to current anatomical based technology.

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Improved Detection of Cancer Genes from Pan-Cancer Genome Sequencing Data

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The volume of available cancer genome sequencing data is growing exponentially and one application of these data is the prediction of novel cancer genes. By using patterns of protein-coding somatic mutations to predict genes that underpin tumorigenesis, new avenues for experimental research and potential drug targets can be identified. Several methods to address this task have been published and often use non-redundant rationales. Although it is likely that a combination of methods would yield better predictions, performance criteria and strategies for integrating them are underdeveloped. In this study we compare the ability of established methods to predict a literature-based cancer gene panel, develop new methods to address shortcomings, and integrate these methods into a single random forest model. We begin by first testing existing methods with a pan-cancer dataset consisting of 1.7 million mutations. In general, we found that existing methods less effectively detect oncogenes than tumor suppressor genes (TSG). This is a considerable concern since oncogenes are more likely to serve

as drug targets. To address this weakness, we developed several new methods designed to isolate oncogenes as well as tumor suppressors, and to separate these two classes from one another. In particular, our new method based on sample mutation frequency detects TSGs and oncogenes with AUROCs of 0.90 and 0.89, respectively, while our method based on frequencies of truncating events separates TSGs from oncogenes with an AUROC of 0.92. Given the complementary of these methods, we reasoned that a model integrating several methods would provide even stronger performance. We developed a Random Forest model that relies on six individual methods. This model identifies both TSGs (AUROC=0.98) and oncogenes (AUROC=0.90) better than any individual method tested and suggests GPS2, HDAC2, HIST1H1E, EPHA5, STGAL2, CACNG3, NXF1 as potential new cancer genes.

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BMI Associations with Graft Failure

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Objective: Obese patients have recently been shown to have mortality benefit from renal transplantation. However, graft failure is less well characterized in this cohort. Risk factors for graft failure include acute rejection and proteinuria. Leveraging the Scientific Registry of Transplant Recipients (SRTR), we sought to determine whether BMI have any significant associations with proteinuria, acute rejection, graft failure, and time to failure. **Design And Methods:** We utilized data from the SRTR, which includes data on all donor and transplant recipients in the United States. 339,020 unique patients were annotated in our dataset, ranging from 1/10/1987 to 3/2/2013. After adjusting for missing variables, a total of 275,523 recipients were analyzed. Demographics at the first visit, indicators of any elevated urine protein and acute rejection, and time to failure were analyzed. Continuous variables were summarized by median and interquantiles, whereas categorical variables were by frequency and percentage. Differences across BMI were tested by KruskalWallis test and Chisquared test for continuous and categorical variables, respectively. **Results:** Adverse outcomes were significantly associated with increasing BMI, p≤0.0001. Obese category recipients were significantly more likely to have proteinuria (obese 35.8% versus normal 26.1%, p=<0.0001). Obese categories also had increased incidence of acute rejection and graft failure, and decreased time to graft failure p=<0.0001. We also found significant differences in outcomes amongst different ethnicities, p≤0.0001. African Americans were more likely to be obese and they had higher rates of complications/adverse outcomes compared to Caucasians. **Conclusions:** Our findings suggest that increasing BMI, particularly in obese categories and ethnic background can influence susceptibility for graft rejection/failure. To complement these epidemiological insights, understanding the role of molecular pathways in obesity and molecular differences amongst ethnicities may enable for prevention of graft failure in these patients. Ongoing prospective studies are integrating microbiome analyses coupled with metabolic and molecular profiling from renal transplant patients from across a spectrum of BMI and ethnicities to help us gain a comprehensive understanding of drivers.

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A Fibrinogen-Derived Molecule Generated by Diverse Proteinases that Primes Fungistatic Immunity

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Background: Fibrinogen proteolysis within the airway lumen yields a novel ligand that drives the expression of allergic airway disease and antifungal immunity by acting on Toll-like Receptor 4 (TLR4). Inhibition of airway proteinases or the absence of TLR4 attenuates these processes both *in vitro* and *in vivo*. However, it remains unclear which proteolytic product of fibrinogen acts on TLR4 and what types of proteinases are able to induce this antifungal effect. **Methods:** Fibrinogen was incubated with thrombin, the proteinase from *Aspergillus melleus* (PAM), or a proteolytically active extract derived from the dust mite *Dermatophagoides farinae* (DF) to generate three types of fibrinogen cleavage products (FCPs). FCPs were subsequently analyzed by SDS and native PAGE to determine the size of the fibrinogen fragments produced. Fungistasis was assayed *in vitro* by incubating FCPs with murine splenocytes or bone marrow-derived macrophages (BMDMs) for 24 hours, followed by the addition of fungal spores from *Aspergillus niger*. After 18 hours the number of fungal mycelia were quantified and compared to controls. **Results**: Thrombin, PAM, and DF FCP preparations all contained fibrinogen fragments in the range of 160-250 kDa. Whole fibrinogen (340 kDa) and known fibrinogen degradation products (fibrinopeptides (~1.5 kDa) and fibrinogen domains D and E (<150 kDa)) showed no fungistatic activity as defined by a significant reduction in fungal growth in treated as compared to sham controls. In contrast, all FCP preparations primed murine splenocytes to significantly inhibit fungal growth *in vitro* (p<0.05). Studies were also performed using murine bone marrow-derived macrophages that yielded similar results. **Conclusions:** In the presence of diverse proteinases, human fibrinogen degrades to produce similar cytokine-like molecules that potently induce TLR4-dependent anti-fungal immunity from macrophages and splenocytes. Given the unique molecular size of these fragments, we propose the existence of a novel fibrinogen-derived TLR4 ligand with potent immunostimulatory activity.

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Seeking Major Genetic Modifiers of Thoracic Aortic Aneurysm Severity

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Rationale: Thoracic aortic aneurysm (TAA) is characterized by dilation of the proximal aorta and confers risk of dissection and sudden cardiac death. Reduced penetrance of TAA has been consistently observed in familial TAA whereas variable expressivity is common in Marfan syndrome (MFS). Because the natural history of TAA is poorly understood and the disease is clinically silent, there is a need to identify those patients at risk for disease progression at an early stage in order to optimize management. **Objective:** Our objective is to identify major genetic modifiers that regulate TAA severity using whole exome sequencing and an extremes of phenotype approach in patients with known disease causing TAA variants (genotype positive). We hypothesize that there are genetic variants that increase the risk of severe TAA. We postulate that the cumulative burden of variation in pathways of vascular smooth muscle cell contraction, elastic fiber assembly, and TGF-beta signaling, genes that are known to contribute to disease pathogenesis, exhibits a non-random molecular signature that predicts disease severity. **Methods and Results:** Exome analysis was performed in three pedigrees in which genotype positive first degree relatives exhibited divergent TAA severity. TAA severity was classified as severe (Z>+6), mild (+2<Z<+4), or "at-risk" (genotype positive and Z<+2). Rare variants, defined as variants with a minor allele frequency < 1% or absent from dbSNP, were identified that were not shared between family members with divergent phenotypes. There were on average 394 heterozygous rare variants exclusive to the severe TAA group (potentially harmful variants). Conversely, there were on average 471 heterozygous rare variants in the mild or at-risk for TAA patients that were not present in their respective family members with severe TAA (potentially protective variants). Seventy genes containing protein-altering variants were shared across pedigrees and exclusive to severe TAA patients. Conversely, there were 115 variant-containing genes shared across pedigrees and exclusive to patients with mild TAA or at risk for TAA. Utilizing the ToppGene gene list prioritization bioinformatics application, elastin (*ELN*) was ranked 2nd among the latter list of 115 potentially protective genes. A test cohort (n=44) consisting of patients with MFS or familial TAA is being investigated for the presence of highly prioritized variants. **Conclusions:** Exome analysis using an extremes of TAA phenotype approach identifies variants specific to severe versus mild TAA. Identifying genotype-based predictors of TAA severity may facilitate risk stratification at an early stage of disease.

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A Biomechanical and Clinical Comparison of Midshaft Clavicle Fixation Performed with Either Two or Three Screws on Each Side of the Plate

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Objectives: Plate fixation with six cortices of purchase (three screws) on each side of the fracture has been the standard of care when operatively treating displaced midshaft clavicle fractures. The use of locking plates and screws may afford equivalent biomechanical strength and clinical outcomes with only four cortices of purchase (two screws) on each side of the fracture. The purpose of this study is to compare the biomechanical and clinical performance of three-screw and two-screw constructs for displaced midshaft clavicle fractures. **Methods:** Biomechanical studies employing cantilever bending, deemed most physiologic of midshaft clavicle fracture models, have demonstrated that plate bending and fracture through the end screw hole are the common modes of failure. After simulating midshaft fractures in 10 pairs of embalmed cadaveric clavicles, the biomechanically inferior lateral fragments were randomly assigned to plate fixation with either three non-locking screws or two locking screws. Cyclic tensile loads were applied for 5 minutes along the long axis of the clavicle. Then, the constructs were loaded to failure with pullout forces applied parallel to the long axis of the screws. Additionally, we retrospectively identified 41 patients who had midshaft clavicle fractures surgically repaired with a minimum follow-up of 6 months: 21 patients were treated with three-screw constructs and 20 patients with two-screw constructs. Patient reported outcomes, radiographic time to union, and complication rates were compared. **Results:** Biomechanically, there were no significant differences in cyclic displacement (p=0.17), stiffness (p=0.94), yield load (p=0.65), or ultimate load (p=0.622) between the two groups. Clinically, there were no significant differences in American Shoulder and Elbow Surgeons score (ASES) (p=0.35), Constant score (p=0.34), Visual Analog Scale (VAS) pain score (p=0.34), Single Assessment Numeric Evaluation (SANE) score (p=0.99), or average time to union (p=0.74). Complication rates (painful hardware, non-union, hardware failure) trended toward being higher in the three-screw group (p=0.20). **Conclusion:** Plate fixation of displaced midshaft clavicle fractures with four cortices of purchase using two locking screws performs biomechanically and clinically equivalent to fixation with six cortices of purchase using three non-locking screws. Potential beneficial clinical implications include decreased surgical exposure, morbidity, time, and cost. Particularly, with regards to midshaft clavicle fractures, the reduction of the required number of points of cortical fixation from six to four allows the surgeon, in most instances, to use shorter and non contoured straight plates, eliminating the extra time and technical difficulty typically required to fit longer contoured plates to the variable and complex anatomy of the clavicle.

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Task-Relevant Features Predict Gaze Behavior but not Neural Activity in FEF During Natural Scene Search

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When we visually search for an object, our gaze is attracted to parts of the environment that are statistically similar to that object (target-similar, or "relevant" objects). But how does the brain select eye movement targets based upon relevance? Here we ask whether the frontal eye field (FEF), a prefrontal brain region implicated in eye movement selection, shows a reflection of relevance while searching natural scenes. We use a multiple regression based approach to show that relevance attracts gaze but shows no significant influence on FEF activity. We find that this is not due to low power, and that if a linear influence of relevance on FEF activity exists, it must be small. Instead, FEF activity appears to strongly relate to features of eye movement during natural search. Our findings thus contribute to a growing literature that indicates that the responses of higher level brain areas to natural scenes are remarkably complicated.

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Two Distinct Populations of Ventral Tegmental Area Dopaminergic Neurons Exhibit Unique Response Profiles to Repeated Aversive Events

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Dopaminergic (DA) neurons in the ventral tegmental area (VTA) have been found to be responsive to aversive events. In a combat situation, military personnel are often subjected to repeated traumatic or aversive events. An investigation of how DA neurons in the VTA respond to repeated aversive events may shed light for novel therapy in treating disorders such as post-traumatic stress disorder or generalized anxiety disorder. To accomplish this task, we employ multi-tetrode in vivo recording in freely behaving mice and subject these animals to one hundred trials (10 blocks total, each block consisting of 10 events) of unpredicted shaking—an event that has been shown to induce robust DA neuron response. Furthermore, because identifying DA neurons based on electrophysiological characteristics was reported to be unreliable, we combine optogenetics and pharmacology to insure the positive identification of DA neurons. Only animals with optically identified DA neurons were used in the experiments. In agreement with previous reports, we find a distinct group of VTA DA neurons (type I) that exhibits suppression followed by phasic offset-rebound excitation, and another group (type III) that shows phasic excitation to aversive events. Interestingly, type I DA neurons exhibit a progressive increase in offset-rebound excitation over time. In contrast, type III DA neurons show a habituating, progressive decrease in phasic excitation over time. Here, we demonstrate that there are at least two distinct, optogentically identifiable dopaminergic neurons subtypes in the VTA, and each subtype exhibits unique response profiles to repeated aversive events.

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Combination Therapies of Robot Rehabilitation and Viral Delivery of Brain-Derived Neurotrophic Factor (BDNF) to Lumbar Spinal Cord Promote Large Functional Gains After a Complete Transection SCI in Adult Rats

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Spinal cord injury (SCI) disrupts the normal, healthy architecture of the central nervous system, leading to impaired locomotor function. In the rat model, complete thoracic spinal cord transection at T8-T10 is a common model for studying SCI. In our previous work, using trunk-based robotic rehabilitation and treadmill training, we showed that rats spinalized as neonates can significantly recover locomotor function with robotic intervention at the pelvis, whereas rats transected as adults do not exhibit the same level of recovery. We believe this is due to the absence of autonomous reflex hindlimb stepping patterns in adult transection, resulting in an inability to incorporate and benefit from robot support. Previous work by Boyce and Mendell has demonstrated that use of adeno-associated virus-5 (AAV5) viral delivery of neurotrophic factors, such as BDNF and NT-3, to enable reflex hindlimb stepping in the rat, Boyce and Lemay demonstrated related gains in the cat model. We thus propose a combined treatment approach, using a neurotrophin intervention combined with robot training to induce autonomous stepping and achieve greater locomotor recovery. We prepared two groups of rats (n=4 per group) with microinjections (final volume: 8 microliters) caudal to transection site into the ventral horn of the spinal cord: one group receiving AAV5-BDNF and another receiving a sham AAV5 virus expressing green fluorescent protein. Following post-operative recovery, animals were treadmill trained with robotic pelvic rehabilitation therapy for six weeks. We used the Antri, Orsal, and Barthes (AOB) bipedal stepping scale and robot data measuring the interactive force between the rat and the robot to characterize improvement and recovery of locomotor function. We found that animals that received AAV5-BDNF and robot-assisted treadmill training showed significantly improved recovery over the course of training in both AOB ($p<<0.001$) and overall robot interactive force ($p<0.001$), compared to those that received sham virus, which did not. Comparing across both groups, locomotor recovery assessed on the final day of therapy also showed significant improvement in the experimental group, as compared to the control group, with respect to AOB scores ($p=0.0001$) and robot interactive force ($p=0.0002$). This work provides a foundation upon which to investigate further combinations of biological and bionic therapies for treating SCI. This work is sponsored by the Craig H. Neilsen Foundation and the NS 54894.

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Conditionally Reprogrammed Nasal Epithelial Cells Illustrate a Model for Investigating Allergic Rhinoconjunctivitis

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Background: Loss of epithelial cell integrity plays a key role in the development of allergic rhinoconjunctivitis (AR), a highly prevalent condition. However, a means to evaluate epithelial cell changes in a non-confounded manner in large numbers of samples poses a formidable challenge. The use of an allergen challenge chamber (ACC) provides for a controlled exposure of allergen and also mitigates confounders in the natural environment (e.g. variable concentration of allergen). **Objective:** Capitalizing on the ACC and novel epithelial cell reprograming technologies we sought to develop a bio-bank of epithelial cells from allergic and non-allergic individuals exposed to house dust mite (HDM) in an ACC. **Methods:** Individuals sensitive (HDM+, n=25) vs. non-sensitive (HDM–, n=15) to HDM were exposed on 4 consecutive days for three hours each to HDM extract (~170 ng/m^3). Nasal swabs were collected before the start of the final ACC exposure and were used to generate conditionally reprogrammed nasal epithelial cells (CR-NECs) *in vitro* using methods of Suprynowicz *et al*. (*PNAS*, 2012). CR-NECs were deprogrammed to generate primary NECs and phenotypically characterized by analyzing the constitutive expression of chemokine receptors and cytokine production. Whole genome transcriptomic expression was determined by RNA-Seq of deprogrammed NECs (in 1 HDM+ and 2 HDM–), before and after stimulation with HDM (10 µg/mL) *in vitro*. **Results:** The expression of cytokeratins 5/6/8/18 and biomarkers (Integrins α1/β6, Notch-1 and CD44) respectively confirmed epithelial origin and the reprogrammed state of NECs (at passage (P) 1). At P2, deprogrammed NECs expressed significantly higher levels of CCR4 and lower amounts of EpCAM compared with CR-NECs (P<0.05). Constitutively, deprogrammed NECs from HDM+ compared with HDM– individuals expressed higher levels of EpCAM and CCR4 levels, and produced higher levels of IFNγ and IL-23 but lower levels of IL-8. 242 genes were differentially expressed when comparing resting vs. HDM–stimulated deprogrammed NECs (FDR < 0.05, 160 up-regulated, 82 down-regulated). The up-regulated genes were primarily involved in interferon signaling, pattern recognition, IL-17 signaling and antigen presentation. **Conclusion:** Conditional reprogramming of NECs followed by deprogramming provides a potentially unlimited source of primary epithelial cells that preserve immunologic characteristics. Preliminary evidence suggests that immunological differences in the NECs from HDM+ vs. HDM– individuals are preserved. This is reflected by higher levels of CCR4 which has been implicated in the pathogenesis of AR. We believe our approach provides an innovative model system to further evaluate the role of epithelial barrier integrity in AR pathogenesis.

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Nuclear Receptor Nur77 Decreases Osteoclast Differentiation by Promoting NFATc1 Degradation via Ubiqutin E3 Ligase Cbl-b

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Osteoclasts are essential for skeletal remodeling, repair and regeneration. They remove old or fractured bone to allow new bone matrix to be deposited by osteoblasts. However, when osteoclast activity becomes excessive, bone degenerative diseases such as osteoporosis, inflammatory arthritis and cancer bone metastasis occur. Osteoporotic fractures alone affect 1 in 3 women and 1 in 5 men over the age of 50. Despite osteoclast's common involvement in human diseases, we only have 2 classes of clinically approved osteoclast specific inhibitors, bisphosphonates and denosumab (anti-RANKL antibody). Furthermore, these drugs have significant limitations and side effects such as osteonecrosis of the jaw (ONJ) and renal toxicity. In our research, we found that the expression of the nuclear receptor Nur77 is up-regulated during osteoclast differentiation. In addition, Nur77 KO mice exhibited increased osteoclast activity and bone resorption, which resulted in osteoporotic bone. Similarly, we found that human patients with osteoporosis and rheumatoid arthritis express lower level of Nur77 compared with control patients, possibly due to elevated osteoclast activity. Mechanistically, Nur77 can inhibit the activity of NFATc1, the master regulatory transcription factor in osteoclastogenesis, by promoting its degradation. Over-expression of Nur77 decreased NFATc1 protein level without affecting its mRNA level, while loss of Nur77 in osteoclasts prevented NFATc1 from being degraded. We further discovered that Nur77 can transcriptionally up-regulate Cbl-b, an E3 ligase that has been shown to mediate NFATc1 ubiquitination and degradation. Over-expression of Nur77 increases Cbl-b expression, while Nur77 KO osteoclasts have decreased Cbl-b level. In addition, luciferase assay reveals that Nur77 can activate Cbl-b promoter. Mutating the Nur77 response elements (NurRE) in Cbl-b promoter considerably diminished its activity, and deleting the DNA binding domain (DBD) of Nur77 completely abolished its ability to up-regulate Cbl-b. Coincidentally, NFATc1 also emerged as a Nur77 up-stream regulator. Over-expression of NFATc1 induced Nur77 mRNA expression, whereas treating cells with cyclosporine A, an NFATc1 activity inhibitor, significantly reduced Nur77 levels. Bioinformatic analyses reveal that Nur77 promoter contains several NFATc1 response elements, and ChIP and luciferase assay confirmed that NFATc1 can bind and activate Nur77 promoter. These results support a novel self-limiting loop in which NFATc1 induces its own degradation by activating the Nur77-Cbl-b loop. It is our hope that with the understanding of how Nur77 regulates NFATc1 and osteoclast differentiation, we can develop novel and better therapeutic options to treat bone degenerative diseases.

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Heat Shock Proteins Protect Skeletal Muscle Against Frostbite Injury

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Backgound: Incidence of frostbite, a freeze injury which can result in severe soft tissue and skeletal muscle damage, is increasing in the general population and remains a challenging disease entity frequently encountered by military personnel. Tissue damage is caused not only by immediate cold-induced cell death, but also from the local inflammatory processes and ischemia that follow the initial insult. Previous studies indicate that heat shock proteins (hsp) are able to protect against oxidative stress and inflammation. **Results:** In the present study, we examined the effects of the heat shock protein inducer 17-Dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG), which was administered locally within 45 minutes following frostbite injury. Our results show that rat hind-limb muscles injected with 17-DMAG following frostbite injury exhibit less inflammatory cell infiltration compared to control rat hind-limb muscles. In agreement with this observation, we found that the increased heat shock protein expression results in a decrease in inflammatory cytokine expression. We also found that administration of 17-DMAG after frostbite injury is able to preserve muscle function. Our results therefore suggest that 17-DMAG protects skeletal muscle if administered shortly after frostbite injury. **Conclusion:** We conclude that compounds such as 17-DMAG, which induce heat shock proteins, are able to preserve skeletal muscle function and structure if injected within 45 minutes after frostbite injury. Our studies provide the basis for the development of a potential adjunctive therapy in the treatment of frostbite injury. This research was supported by an award from US Army Medical Research and Material Command.

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Identification and Characterization of an MC4R Specific Negative Allosteric Modulator

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Aberrations of the hypothalamic orexigenic signaling network underlie the pathophysiology of cancer cachexia. Rodent models of cachexia are characterized by activation of the melanocortin 4 receptor (MC4R) neurons in the paraventricular nucleus by excessive α-Melanocyte stimulating hormone (α-MSH) secretion. The endogenous inverse agonist of MC4R, Agouti Related Peptide (AgRP) that normally counters the effects of α-MSH is decreased in cachexic rodents. The end result of this neurohormonal alteration is decreased food intake and increased energy expenditure, the hallmarks of cancer cachexia. Therefore, pharmacological dampening of MC4R signaling remains an untapped target for the treatment of anorexia-cachexia syndrome (ACS). In this study, we identify and characterize 47 potential negative allosteric modulators (NAMs) of the MC4R by screening 40,000 compounds. Compounds were screened in HEK cells expressing a cAMP sensitive modified luciferase reporter gene and the MC4R. Hits were counter screened against β2AR to determine specificity. Selectivity studies revealed a highly selective hit VU58711 with a pA2 of 6.22 indicating submicromolar affinity. This compound was inactive at the both β2AR and MC3R demonstrating excellent receptor specificity, and the viability of targeting allosteric sites of the MC4R. Together, these results demonstrate tractable hits with potential for further development as therapeutic MC4R inhibitors for the treatment of cancer cachexia.

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Microscopic Image Guidance and Real-Time Monitoring of Thermal Therapy in Barrett's Esophagus

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The term "image-guided therapy" traditionally refers to the use of *macroscopic* imaging modalities (such as CT, MR, or ultrasound) to guide therapeutic interventions. Here, we introduce a new paradigm of *microscopic* image guidance and real-time thermal therapy monitoring based on a novel imaging technology developed in our lab, called optical frequency domain imaging (OFDI). OFDI is a clinically validated diagnostic imaging modality that provides tissue microstructural details at high-resolution (~10um) and has shown significant promise in Barrett's esophagus (BE) screening in our earlier clinical studies. In particular, BE with high grade dysplasia (HGD) is a condition that carries a significant risk of progression to esophageal adenocarcinoma (5-yr survival rate: ~15%). Clinically, thermal therapy, such as radiofrequency (RF) ablation and laser thermal therapy, has become an established treatment modality for early-stage epithelial malignancies including BE with HGD. However, these procedures typically rely on pre-determined energy settings and incorporate minimal guidance using only macroscopic surface features seen through video endoscopy. In this work, we demonstrate the feasibility of using dynamic OFDI measurements previously developed for angiographic applications for real-time thermal therapy monitoring in 2-D (and potentially 3-D), thereby enabling the non-invasive assessment of coagulation zone boundary at high spatial resolution. Conventional thermal therapy monitoring techniques based on temperature and impedance measurements provide only point sampling, while emerging techniques such as MR thermometry or photoacoustic thermography are limited by their spatial resolution. For epithelial applications such as laser therapy in the skin or esophagus, the ability to directly visualize the thermal lesion boundary is critical to the accurate delivery of thermal energy to the target lesion. Here, we evaluate the use of three angiographic reconstruction techniques: (1) speckle variance, (2) intensity-based Doppler variance, and (3) complex differential variance. We showed the ability to accurately delineate the coagulation zone boundary and therapy depth in *ex-vivo* porcine skin and esophagus irradiated with a Thulium CW fiber laser and validated the results with histological analysis. The ability to perform real-time thermal therapy monitoring non-invasively and at high spatiotemporal resolution provides a powerful tool to guide treatment in a vast array of epithelial applications.

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Tumor Necrosis Factor Alpha Receptor 1 Shedding in Sepsis Associated Coagulopathy May Modulate Circulating TNF-α Levels: Potential Role in the Mediation of Pathophysiologic Responses

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Introduction: Tumor necrosis factor alpha (TNF-α) is predominantly secreted by monocytes and macrophages in response to bacterial endotoxins in Sepsis Associated Coagulopathy (SAC). Soluble TNF-α exerts its pathophysiologic responses by binding to specific target cell surface receptors which have been identified as TNF-R1 (P55) and TNF-R2 (P75). It is known that shedding of membranous TNF-R1 plays a major role in the pathophysiology of SAC. This study was designed to investigate the relationship of circulating TNF-α antigen and TNF-R1 in SAC patients. **Materials and Methods:** Banked plasma samples (n=100) from SAC were retrospectively collected from various centers under approved IRBs with a defined criterion. Thirty individual plasma samples from healthy male and female volunteers were used as control. A Randox Technology biochip array (Crumlin, County Antrium, UK) was used to quantify TNF-R1 along with other inflammatory cytokines. TNF-α antigen was quantified using a commercially available ELISA method (R&D Systems, Minneapolis, MN). **Results**: In comparison to normal (0.56 +/- 0.18 pg/ml), the SAC patients exhibited a marked increase (4.00 +/- 2.54 pg/ml) in the TNF-R1 levels. Interestingly, the TNF-α levels in the SAC patients were lower (13.52 +/- 23.51 pg/ml) than normal individuals (40.92 +/-10.47 pg/ml) . Several of the inflammatory mediators such as VEGF, Monocyte Chemotactic Protein, and IL-6 were also increased. **Discussion:** These studies indicate that due to the activation of matrix proteases, membrane bound TNF-R1 shedding is increased in SAC. The circulating TNF-R1may complex with TNF-α thereby resulting in a decreased quantitation of this cytokine in SAC. Furthermore, this complex may serve as a reservoir, releasing sustained levels of TNF-α for an extended period of time, perpetuating the inflammatory response by upregulating inflammatory mediators in this syndrome.

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Therapeutic Potential of Novel Parthenolide Analogs for Enhancing Treatment of Glioblastoma Multiforme

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Purpose: Glioblastoma multiforme is an invariably deadly brain cancer that results in 13,000 deaths annually. Current standard-of-care treatments are ineffective and have changed very little over the past three decades. Thus, there is a great need to discover more effective therapies. Parthenolide is a sesquiterpene lactone, originally isolated from the Feverfew plant (*Tenacetum parthenium*), which has demonstrated potent growth inhibition, cytotoxicity, radiosensitization, and chemosensitization towards many types of cancer, including glioblastoma. However, parthenolide has very low water solubility, limiting its therapeutic potential. In order to overcome this hurdle several new analogs have been synthesized. The goal of this project is to determine which, if any, of these analogs has improved aqueous solubility and similar or improved anti-cancer, radiosensitization, and chemosensitization against glioblastoma cells *in vitro* and *in vivo*. **Methods:** Initially several dozen parthenolide analogs were screened against a panel of 60 human cancer cell lines, which includes several brain tumor lines, in order to exclude ineffective compounds from further study. Those compounds that were found effective in single dose screening 10^{-5} M were then evaluated in five dose screens to determine GI_{50} and LC_{50} values. Based on data gathered from these screens, ten analogs were selected for their specific toxicity against glioblastoma cell lines. These ten compounds were then further tested against 9L-SF cells, a rat glioblastoma line, in order to compare their anticancer activities to that of parthenolide. One compound, dimethylaminoparthenolide (DMAPT) was identified as the lead compound for this study. Further testing has been conducted to evaluate the ability of DMAPT to radiosensitize and induce cell death in the rat and human glioblastoma cell lines and in the 9L-SF tumor model *in vivo*. **Results:** Three analogs were found to have similar or improved cytotoxicity against the 9L-SF rat glioblastoma cell line when compared to parthenolide. However, only one analog, DMAPT, was selected for further investigation because of its high anti-cancer activity against glioblastoma cells and its high water solubility. Additional *in vitro* work demonstrated that DMAPT is able to radiosensitize both human and rat glioblastoma cells. **Conclusions:** DMAPT is able to induce cell death, and radiosensitize both rat and human glioblastoma cells. DMAPT is also non-toxic in the rat model at high doses (100mg/kg), and has potential for improving current standard of care therapy for treating glioblastoma multiforme. Funding provided by *UAMS TRI and NCI grant R01 CA158275;* Additional Funding from *American Foundation for Pharmaceutical Education Fellowship*

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Lupus Risk-Variant Increases pSTAT1 Binding and Decreases *ETS1* Expression

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Background/Purpose: Genetic variants at chromosome 11q23.3, near the gene *ETS1* have been associated with Systemic Lupus Erythematosus (SLE or lupus) in independent cohorts of Asian ancestry. Several recent studies have implicated ETS1 as a critical driver of immune cell function and differentiation, and mice deficient in ETS1 develop an SLE-like autoimmunity. **Methods**: We performed a fine-mapping study of 14,551 subjects using multi-ancestral cohorts, starting with genotyped variants and imputing to all common variants spanning the *ETS1* locus. By constructing genetic models using frequentist and Bayesian association methods, we identified a set of 16 variants that are statistically most likely to be causal. We functionally assessed each of these variants based on their likelihood of affecting transcription factor binding, microRNA binding, or chromatin state. We then employed electrophoretic mobility shift assays and DNA affinity precipitation assays to identify genetic variants potentially having biological function. **Results**: Of the four variants that we experimentally examined, only rs6590330 differentially binds lysate from B cells. Using mass spectrometry, we found an increase in binding of the transcription factor signal transducer and activator of transcription 1 (STAT1) to DNA near the risk allele of rs6590330 compared to the non-risk allele. Western blot analysis and chromatin immunoprecipitation of pSTAT1 in B cells heterozygous for rs6590330 confirmed that the risk allele increased binding to the active form of STAT1. eQTL analysis indicates that the risk allele of rs6590330 is associated with decreased *ETS1* expression in Han Chinese, but not other ancestral cohorts. **Conclusion**: We propose a model in which the risk allele of rs6590330 increases SLE risk by enhancing the binding of pSTAT1, resulting in repression of the *ETS1* expression.

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Amelioration of NADPH-Mediated Stress with Lipoic Acid Reduces Cell Death Following Blast Traumatic Brain Injury

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1.7 million traumatic brain injuries (TBIs) occur each year in the United States. Recent evidence suggests that repetitive TBIs can lead to chronic neurodegenerative changes over time. Currently, available pharmacologic options for the treatment of acute neurotrauma are limited. Oxidative stress is an important secondary mechanism of injury that can lead to cellular apoptosis and behavioral changes such as impulsivity. Utilizing a clinically relevant and validated rodent blast model, we investigated how NADPH oxidase expression and associated oxidative stress contributes to cellular apoptosis following single and repeat blast injuries. Nox4 forms a complex with p22phox following injury, both of which are important subunits of the NADPH oxidase system found within the brain. Using immunohistochemical-staining methods, we found a visible increase in Nox4 following single blast injury in Sprague Dawley rats. Interestingly, Nox4 was also increased in post-mortem human samples obtained from athletes diagnosed with chronic traumatic encephalopathy (CTE). Nox4 activity led to an increase in superoxide formation. Alpha lipoic acid, an oxidative stress inhibitor, decreased Nox4 when given i.p. 10mg/kg 5-minutes after blast exposure. Lipoic acid also significantly decreased the subsequent formation of superoxide. Lipoic acid additionally increased the anti-apoptotic markers Bcl-2 ($t = 3.079$, $p<0.05$) and heme oxygenase 1 ($t = 8.169$, $p<0.001$) compared to blast injured animals when measured with immunoblotting. Two weeks following the last blast exposure (6 blasts total every other day for 2 weeks), the lipoic acid treatment group had significantly reduced pro-apoptotic markers Bax ($t=4.483$, $p<0.05$), caspase 12 ($t=6.157$, $p<0.001$), and caspase 3 ($t=4.573$, $p<0.01$) compared to blast injured animals without treatment when measured with fluorescent immunohistochemistry. Lipoic acid treated animals also had significantly reduced tau hyperphosphorylation indicated by reduced CP-13 and PHF staining. Tissue from the National Football League player also had increased Bax expression ($t=5.424$, $p<0.01$) compared to control. A significant difference in impulsive-like behavior between experimental animal groups was seen as well, based on time spent in the open arm of the elevated plus maze, 7 days after repetitive blast injury ($F(2,21)=4.327$, $p<0.05$). Rats exposed to repetitive blast displayed increased time in the open arm compared to control ($t=3.632$, $p<0.05$), but lipoic acid given 5 minutes after each blast ameliorated this impulsive-like behavior ($t=3.573$, $p<0.05$). TBI can cause debilitating symptoms, disability, and psychiatric disorders. Secondary mechanisms of injury, such as oxidative stress, are ideal targets for neuropharmacologic intervention. Alpha lipoic acid warrants further investigation as a therapeutic for the treatment of acute neurotrauma and prevention of chronic neurodegeneration.

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APOL1 is an Innate Immunity Effector that Induces Stress Autophagy by Interacting with the SNARE Protein, VAMP8

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Background: Apolipoprotein L1 (APOL1) prevents African Sleeping Sickness, a trypanosomal disease. Some trypanosome species express a serum resistance associated protein (SRA) that blocks APOL1's trypanolytic activity. Genetic variants in the APOL1 SRA-binding domain (G1-S342G & I384M and G2-Del N388 & Y389) circumvent parasitic resistance, but associate with non-diabetic kidney diseases in African American patients. Most individuals with *APOL1* risk genotypes do not develop kidney disease, suggesting a "second hit" is necessary. APOL1 function in humans is not known. We hypothesized that SRA-binding domain of APOL1 interacts with a human protein(s) orthologous to SRA and that identification of APOL1's binding partner(s) would reveal its function(s). **Methods:** APOL1 and VAMP8 localization and expression was examined in cultured cells and normal human kidney sections. Immunoprecipitation, pull-downs and surface plasmon resonance (SPR) were performed using standard methods. Computational methods were used to generate three dimensional models of APOL1 wild type (G0) C-terminus:VAMP8 SNARE domain complex and conformational changes were assessed with all-atom Molecular Dynamic (MD) simulations. Autophagy assays and VAMP8 shRNA knockdown were done on 293T cells. **Results:** We used a structural homology search to identify human protein similar to trypanosomal SRA protein. We identified the endosomal/lysosomal SNARE protein, VAMP8, as a human protein candidate that interacts with the carboxy-terminal portion of APOL1. Confocal microscopy demonstrated that APOL1 and VAMP8 colocalized in the podocyte in normal human kidney. Immunoprecipitation and pull down assays confirmed the interaction between VAMP8 and APOL1, which was attenuated by the G1 or G2 variants. Interaction analysis between APOL1-G0 and VAMP8 with SPR showed a KD between 30 and 100μM, which was reduced significantly in case of the mutants. All-atom MD simulations (40 ns) showed an "open" conformation adopted by C-terminal domain of APOL1-G0 in contrast to the "closed" hairpin helix of G1 and G2 variants. MD simulations suggested that APOL1 G0 C-terminus:VAMP8 SNARE domain interaction is plausible with a 3:1 stoichiometry while a monovalent (1:1) interaction is unfavorable. *In vitro* and *in vivo*, APOL1 induced autophagy, which was attenuated by deletion of the VAMP8 binding domain. VAMP8 knockdown created an autophagic block in cells transiently transfected with APOL1-G2, but not in cells transfected with APOL1-G0. **Conclusions:** G1 and G2 variants of APOL1 cause a conformational change in the carboxy-terminal domain of APOL1 resulting in a "closed" conformation that limits interaction with VAMP8. Interaction of APOL1 C-terminus with VAMP8 regulates autophagy in a variant dependent manner. Attenuated APOL1:VAMP8 interaction likely prevents execution of adaptive stress responses that prevent kidney injury.

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Deciphering Inflammation Through Structural Studies of Calcium-Independent Phospholipase A2β

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Calcium-independent phospholipase $A_2\beta$ ($iPLA_2\beta$), through hydrolysis of the sn-2 position of phospholipids, generates inflammatory mediators and is involved in the regulation of calcium homeostasis in cells. Altered $iPLA_2\beta$ activity has been linked to multiple diseases such as myocardial ischemia, diabetes, muscular dystrophy and cancer. Mutations in the protein have been identified in neurodegenerative diseases such as Parkinson's and neuroaxonal dystrophy. We hypothesize that protein-protein interactions of $iPLA_2\beta$ and their associated signaling pathways may explain how it plays a role in a wide variety of diseases. However, the mechanisms by which this enzyme is activated and regulated at the molecular level are poorly understood due to the lack of structural and detailed functional information. Calmodulin (CaM) is proposed to tonically inhibit $iPLA_2\beta$ in the cell until activation occurs. However, the known CaM recognition motifs identified in $iPLA_2\beta$ alone have low affinity for calmodulin, in contrast with the relatively high affinity of the full-length proteins (K_D~150nM), suggesting the presence of additional structural determinants. To quantitatively determine the enzymatic parameters of $iPLA_2\beta$, we have developed a sensitive continuous activity assay using fluorescent phospholipids, an advantage over radiolabeled methods. The IC_{50} of calmodulin (108nM) is in agreement with the binding constant, and supports the idea of physiological inhibition. Additionally, we have determined that $iPLA_2\beta$ exists as a dimer in solution and when bound to CaM. Taken together, these data suggest that the oligomeric state is likely an important feature of the enzyme's regulation. The assay will be essential in evaluating the sources of enzyme activation or characterizing disease mutants. This work enables detailed study into the protein's structure-function relationships, and will provide critical insights for the development of novel inhibitors and modulators of $iPLA_2\beta$ activity and inflammation.

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The Development of a National Physician-Scientist Registry in Canada

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The Clinician Investigator Trainee Association of Canada / Association des cliniciens-chercheurs en formation du Canada (CITAC/ACCFC) represents over 300 MD+ (MD/PhD, MD/MSc) and Clinician-Investigator Program trainees across Canada. Our vision for CITAC/ACCFC for the next decade is for us to become a think-tank in physician-scientist training. In 2013, CITAC/ACCFC published two manuscripts that examined the demographic breakdown and satisfaction levels of physician-scientist trainees in Canada. Through our efforts, we hope that trainees will be better represented by us and their institutional program directors. Our current membership database represents the most comprehensive collection of its kind for Canadian trainees, but we have yet to capture every trainee in the country. Given the large Canadian trainee population, we are afforded with the opportunity to conduct population-based outcome studies. Consequently, our current initiative is in collaboration with the Canadian Post M.D. Education Registry (CAPER), which will enable us to obtain trainee outcome data on the location of their practices (academic *vs.* non-academic). In consolidating the current CITAC/ACCFC and CAPER datasets, we will have the capacity to perform the world's first nation-wide study of physician-scientist trainees. The gathered data will also establish the infrastructure for follow-up studies relating to number of publications, awards, tenure status, and other outcome measures that define success in academic medicine. We anticipate our current framework for the generation of a national physician-scientist registry to serve as a working model that may easily be replicated in other other nations, with the long-term goal of performing international comparisons between outcome measures of different physician-scientist training systems.

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Programmed Self-Assembly of a Peptide-MHC Dynamic Anchor for Antigen-Specific Immune Modulation

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A technology to elicit the full activation of any desired population of T cells in the body—particularly those that possess low avidity against target antigen—would pave the way to the design of new types of vaccination for intractable infectious diseases or for cancer. Here we report such a technology based on positive feedback-driven, programmed self-assembly of peptide-MHC (pMHC) directly on the membrane of cognate T cells. Our design capitalizes on the unique features of the protein annexin V (ANXA5), which behaves as a 'dynamic anchor' that senses and reacts to specific microenvironmental cues. The dynamic anchor—in a concerted and synergistic manner—couples the early onset of TCR signaling by cognate pMHC with a surge in pMHC-TCR affinity, with repeated pMHC encounter, and with widespread TCR crosslinking. In our system, the dynamic anchor is linked to pMHC and firmly engages the plasma membrane of cognate T cells upon (and only upon) the early onset of TCR signaling. This anchor in turn exerts a mechanical force that stabilizes interactions at the TCR-pMHC interface. Furthermore, once the dynamic anchor attaches to these T cells, it facilitates repeated, serial pMHC encounter and quickly arranges into uniform 2D matrices, thereby prompting TCR crosslinking. We found that fusion of ANXA5 to pMHC augments activation of cognate T cells by several orders of magnitude (>1,000-fold), bypasses the need for costimulation, and breaks tolerance against a model self-antigen *in vivo*. Our study opens the door to the application of synthetic, feedback-driven self-assembly platforms in immune modulation.

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Intrinsic Changes in Striatal Plateau and Low Threshold Spiking Interneurons in Experimental Parkinson's Disease: Implications for Synaptic Plasticity

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The striatum is the largest member of the basal ganglia, a group of nuclei which associate actions with motivation. The striatum plays an especially critical role in these tasks, integrating converging glutamatergic and dopaminergic inputs that are subsequently processed by complex intrastriatal microcircuits which serve to link these actions to outcomes. Striatal interneurons are a key part of these microcircuits. Though they make up <10% of all cells within the nucleus, their modulation of striatal projection neurons (SPNs) intrinsic and synaptic excitability is critical to ensuring the proper message is output from this nucleus. Striatal interneurons are heterogeneous groups of cells that have only recently been described in any detail, due to past difficulties in their identification and the nucleus' anatomical complexity. One of the most ill defined groups of cells are the striatal plateau and low threshold spike interneurons (PLTSIs). These cells make inhibitory contact on distal dendrites of SPN, but also express neuropeptide Y (NPY), somatostatin (SST), and neuronal nitric oxide synthase (nNOS). Our group has recently shown that the latter two compounds exert a powerful influence over SPNs; nitric oxide (NO) in particular, induces synaptic long term depression (LTD) in SPNs. Because LTD is altered in diseases of the striatum, such as Parkinson's disease (PD), the continued study of this newly described form of plasticity in an animal model of PD should prove beneficial towards understanding striatal adaptations to altered dopamine (DA) levels. Our preliminary data suggests that NO mediated LTD is, in fact, compromised in models of PD which contributes to overall SPN dysfunction. Additionally, in a mouse model of PD, we have found adaptations of glutamatergic inputs onto PLTSIs, alterations in how these

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inputs modulate PLTSI firing, and a decreased response of PLTSIs to agonists or antagonists of their DA receptors (DAR). This work is the first systematic characterization of the adaptations of PLTSIs, and their immediate effects on SPNs, in a model of PD. These observations could prove critical to the development of novel therapeutic strategies as there are still no drugs that can delay or provide neuroprotection to patients afflicted with PD.

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Cellular Microparticles as Predictive Markers for Adverse Events in Patients with Implanted Ventricular Assist Devices

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Purpose: Implantation of continuous flow ventricular assist devices (CF-VADs) for advanced heart failure management has become more frequent but patients remain at risk for complications directly associated with the therapy. Cellular microparticles (MPs) are formed as a result of cellular activation or damage and may play a role in mediating hemostatic or inflammatory alterations. The altered shear stress on blood induced by the CF-VAD may be a further source of MP generation. Our study sought to determine whether alterations in blood MP levels are observed prior to the occurrence of common adverse events in patients with implanted CF-VADs, such that MP levels could serve as predictive markers of imminent complications. **Methods**: Blood samples were collected peri-operatively and during clinic visits, until transplant or expiration, from 30 consented patients implanted with the HeartMate II LVAD (Thoratec). Whole blood specimens collected in 3.2% citrate were centrifuged for platelet poor plasma then ultracentrifuged (20,000 x *g* for 90 minutes) to pellet MPs. Aliquots of the MP rich samples were stained with PKH67 (to identify MPs from biological membranes) then separated into five tubes containing either: Tyrode's buffer (control), CD41-PE (platelets), CD45-PE [leukocytes (WBCs)], CD146-PE [endothelium (EC)] or CD235-PE [erythrocytes (RBCs)]. Samples were analyzed on a Beckman EPICS XL flow cytometer to quantitate MPs using an in-house established and validated assay. Six healthy individuals served to establish normal MP levels. A database of clinical events in the consented patients was created from medical chart reviews by the Heart Failure Clinic. **Results**: With a mean follow-up of 291±91 days (52-330 days), thrombosis and elevated LDH have occurred, thus far, in three patients. CD41+ [2-6 standard deviations (SD) above normal], CD45+ (2-5 SD above normal), CD+146 (5-8 SD above normal) and C235+ (2-5 SD above normal) MPs were observed to be consistently elevated for weeks to months preceding the diagnosis. In these patients, the MPs were within 1 SD of normal for the first two months following implant. Comparatively, the MP levels in patients without thrombosis or elevated LDH events remained within or near normal range throughout. Other analyzed complications, hemolysis and bleeding events, were not as clearly associated with sustained elevations of MPs. **Conclusion**: In CF-VAD patients, quantitation of MP levels has the potential to be a clinically useful assay to predict thrombotic events, amongst other adverse events, in a timely manner allowing for early intervention and correction.

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Combined Digital Breast Tomosynthesis and Near-Infrared Tomography for Breast Lesion Characterization

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Annual screening mammograms are recommended for all women above 40 years of age for early detection of breast cancer, and are known to improve cancer mortality rates. However, mammography is hampered by low sensitivity and a high rate of false positive results that can lead to invasive and possibly unnecessary biopsies. Digital breast tomosynthesis (DBT) is a recently approved screening method that takes multiple X-ray images at different positions relative to the breast, rendering excellent three-dimensional anatomic detail of the breast. Near-infrared spectral tomography (NIRST) uses multiple wavelengths of near infrared light to probe the metabolic status of the tissue. Primary light absorbers in this regime include oxy and deoxygenated hemoglobin, water and lipids. Hence, using NIRST it is possible to quantify blood content, oxygen saturation, water and fat levels non-invasively. The presence of malignancy can alter the metabolic properties of local tissues. When used alone, NIRST is hampered by low spatial resolution. We aim to show that noninvasive differentiation between malignant and benign lesions can be more accurately obtained by combining spatial information from DBT and functional information from NIRST in a single exam. To this end, the NIRST hardware has been integrated into an existing DBT unit, allowing completely co-registered X-ray and optical data. Over 50 women have been imaged to date, with two-four scans per patient. This includes 32 normal subjects and 25 women with abnormal mammographic findings on screening exam. Tissue metabolic markers were analyzed with respect to patient demographics such as body mass index, breast density and composition, breast thickness, bra cup size and breast compression to demonstrate the system's capabilities to discern physiological variations of normal subjects. Additionally, for women with breast lesions, NIRST properties were correlated with biopsy pathology results that included a range of benign and malignant lesions. Pathology, radiologist assessment of DBT images alone and NIRST/DBT combined results were compared for preliminary assessment of sensitivity and specificity improvements when NIRST is utilized as an adjunct to DBT imaging for detection of breast cancers. The addition of NIRST imaging to DBT could aid in clinical decision making and decrease the number of biopsies which are performed with negative results, improving patient experience.

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A Multiplexed Fluorescence Microscopy Method (MultiOmyx™) to Identify Proteomic Biomarkers of (18)F-fluorodeoxy-glucose ((18)FDG) Uptake in Breast Cancer

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Background: Multi-parameter measurements of protein expression at the single cell level have been explored in hematological malignancies, but remain challenging for solid tumors. In this project, we applied a recently developed hyperplexed fluorescence microscopy method (MultiOmyx™) (PMID23818604) to examine the expression of 27 proteins (including segmentation markers) in 26 breast cancer patients who underwent (18)F-fluorodeoxy-glucose ((18)FDG) - PET imaging prior to tumor resection. **Methods:** For each patient, we stained a single 5 µm section with antibodies against selected members of the RTK-PI3K-pathway, the glycolysis pathway, hormone receptors, tumor cell proliferation markers, markers of hypoxia, and angiogenesis. Twenty-eight to thirty representative fields of view (FOVs) were histopathologically reviewed and annotated to distinguish invasive ductal carcinoma (IDC), DCIS, benign tissue, and FOVs with mixed histology. Subsequent analyses focused on 390 FOVs with invasive ductal carcinoma, each comprising 300-500 cells. Automated image segmentation into cell types and subcellular compartments was based on staining with additional markers (NaK-ATPase, S6, DAPI and pan-cytokeratin). **Results:** Staining results with MultiOmyx™ platform correlated closely with the results from CLIA-certified single marker biomarker assays (e.g., ER and PR IHC and HER2 FISH assay) performed independently on the same set of samples. In univariate analysis using linear regression to assess the association between each biomarker metric and FDG uptake, nuclear ER staining, nuclear PR staining, and cytoplasmic PTEN staining were associated with low FDG uptake ($p<.05$). KI-67 staining and Glut1 were increased in tumors with high FDG uptake ($p<.05$). K-median clustering of about 10 Million single cell protein measurements identified eight distinct clusters of protein co-expression that were strongly associated with FDG-PET uptake and represented distinct tumor cell populations. **Conclusions:** Automated hyperplexed measurements of protein expression in routinely collected clinical specimen is feasible and confirmed hormone receptor status and PTEN status as molecular determinants of FDG-PET uptake in locally advanced human breast cancer. This approach appears promising to dissect intratumoral heterogeneity at the level of the proteome and to link this heterogeneity to clinical phenotypes such as in-vivo tumor metabolism or drug response.

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Attenuation of Post-Trauma Hyperglycemia Prevents Acute Kidney Injury in Obese Rats

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Traumatic injury is one of the leading causes of death in the United States, and obese patients have an increased risk of developing post-trauma acute kidney injury (AKI). Acute hyperglycemia after trauma is correlated with worse outcomes, but whether it contributes to AKI in the context of obesity is not known. We hypothesized that blunting the hyperglycemic response would prevent AKI in obese rats. Lower-limb orthopedic trauma was induced in lean (LZR) and obese (OZR) Zucker rats (12wk) by fibula fracture, soft tissue injury, and bone component injection. Plasma glucose levels were measured for five hours after trauma, and glomerular filtration rate (GFR) and plasma interleukin-6 (IL-6) were measured the day after trauma and in non-trauma control rats (all groups $n\geq6$). OZR had higher peak glucose levels after trauma than LZR (275±19 vs 167±13 mg/dL; $p<.05$) as well as prolonged hyperglycemia. Treatment of the OZR after trauma with glucagon-like peptide-1 (GLP-1), an incretin with insulinotropic effects (3µg/kg/hr IV), reduced peak glucose levels to 152±12 mg/dL ($p<.05$). In LZR with trauma, the GFR did not decrease compared to controls (1.44±0.1 vs 1.67±0.2 mL/min/g), while OZR with trauma had significantly decreased GFR as compared to controls (0.96±0.1 vs 1.92±0.1 mL/min/g; $p<.05$). Treatment with GLP-1 in OZR prevented this decrease (1.98 mL/min/g; $p<.05$). IL-6 levels were increased in LZR with trauma as compared to controls (400±114 vs 77±12 pg/dL; $p<.05$), while OZR with trauma had a larger increase (3834±1099 vs 75±6 pg/dL for OZR control; $p<.05$), and GLP-1 treatment decreased IL-6 levels (269±15 pg/dL; $p<.05$). These data suggest that acute hyperglycemia contributes to increased inflammation and AKI development after trauma in obesity. Supported by NIH HL51971, GM104357, and AHA 12SDG12050525, 14PRE17810005

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MAPK Pathway Activated Aberrant Crypt Foci are Associated with Downregulation of Cell Cycle Regulators and Stromal Changes in Matrix and Immune Regulators

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Introduction: Aberrant crypt foci (ACF) are the earliest detectable premalignant lesion within the colon. A majority of ACF harbor somatically acquired mutations to cancer-related genes. ACF may be an indicator for a colonic mucosa at-risk for cancer development, only a small subset of ACF have the potential to progress to malignancy. We believe that the majority of ACF activate growth inhibitory programs that ultimately result in their growth arrest and regression. **Methods:** In order to begin to define the gene expression changes that occur within these early microscopic lesions and their underlying stroma, we have developed a novel method that combines laser capture micro-dissection with targeted transcriptome analysis using RNA-seq. In the following study, gene expression patterns of thirteen human ACF biopsy specimens harboring known oncogenic mutations (*K-ras, B-raf, N-ras,* and *APC*) were compared to matched normal mucosa. Two gene panels were used to assess the status of important cell regulatory pathways: an apoptosis panel comprised of 267 genes and a customized cell senescence panel consisting of 20 genes. **Results:** In the apoptosis panel, the expression of 81 genes were differentially expressed in ACF compared to matched normal mucosa. Expression of HRK, a potent activator of apoptosis, was the most significantly down-regulated gene (6.75-fold, q <0.01) in every ACF when compared to normal mucosa. Inactivation of HRK function occurs in a variety of tumor types, including prostate cancer, astrocytomas and lymphomas. ACF with mutations occurring within the MAP-K pathway (*K-ras, B-raf, N-ras)* showed strong activation of downstream MAPK pathway associated genes and down-regulation of cell cycle regulators (CDK4 (p=0.01), CCNB1 (p=0.04), CCNA2 (p=0.01), and TP53 (p=0.04)). Interestingly, these expression panels were distinct from ACF harboring somatic mutations to *APC*. Analysis of microdissected stroma directly underlying the aberrant crypt structures revealed 25 differentially expressed genes, with dysregulation of several inflammatory mediators (IL2, IL2RA, IL6, TGFB2, BMP4) and extracellular matrix remodeling targets (TIMP1, TIMP2). **Conclusion:** Our findings suggest that MAPK activated ACF may cause significant changes in cell cycle regulation, as well as alterations in stromal extracellular matrix maintenance and inflammation.

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Use of Nano-Formulated Hydroxyurea Enhances Drug Delivery to Pulmonary Arterial Endothelial Cells

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The vasculopathy of sickle cell disease (SCD), characterized by a chronic hemolytic anemia, endothelial dysfunction and recurrent vasoocclusive events, underlies most of the clinical complications. Recent work has suggested that hydroxyurea (HU) enhances endothelial nitric oxide (NO) and reduces adhesion molecule expression which may contribute to its associated observed reduction in vasoocclusive events and improved survival in SCD. However, clinically HU is under-utilized, at least in part related to medication related toxicity, stressing a need for enhanced drug delivery. The use of nano-emulsions, a class of stable emulsions formed by a monolayer of phospholipids and/or biodegradable material such as chitosan, is a mechanism by which to enhance targeted cellular delivery of therapeutics. We hypothesized that a nano-formulation of HU would enhance drug delivery to the endothelium and may be a direct way of targeting endothelial dysfunction in SCD. Nano-formulated HU (nano-HU) was developed using a phospholipid approach and this compound was found to be biologically stable for > 6 months. Human pulmonary arterial endothelial cells (HPAECs) were co-incubated with 25μM, 125μM and 250μM HU, nano-HU, or the empty nanoproduction-emulsification preparation for 48 hours. HPAEC were isolated, and stained with propidium iodide (PI) for cell cycle analysis by FACS. HPAEC treated with 25μM nano-HU demonstrated the highest level of cell viability (55%) and similar levels of S phase inhibition as HPAEC treated with 125μM HU (where 10-20% cell viability was observed). RNA isolation of treated HPAECs was performed and qPCR for VCAM1 and SLX4, a regulator of DNA damage which promotes S phase inhibition was performed. 25μM nano-HU significantly decreased VCAM1 and increased SLX4 expression compared with 25μM HU suggesting increased efficacy at lower levels of toxicity. The impact of nano-HU on endothelial gene expression is currently being evaluated by RNA-seq. Nano-formulation of HU produces a molecule which is highly stable, and able to achieve higher intracellular concentrations with lower rates of toxicity in HPAECs .which provides the opportunity to gain greater understanding of the impact of this medication on the vascular endothelium and the potential for a more targeted and better tolerated delivery of this therapeutic to patients with sickle cell disease.

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Cardiac Troponin TNT1 Mutations Alter Allosteric Interactions in Actomyosin Binding, a Novel Pathogenic Mechanism for Familial Hypertrophic Cardiomyopathy

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Familial Hypertrophic Cardiomyopathy (FHC) is a primary cardiac muscle disorder and a common cause of sudden cardiac death among young people in the field. The majority of FHC mutations in the thin filament regulatory protein hcTnT are found within the TNT1 domain. While this domain has not been structurally resolved, it likely acts as a flexible linker between the two hcTnT functional domains. A highly charged region is found at the C-terminal end of TNT1 (158-RREEEENRR-166) in which this alpha helical domain may unwind to create a flexible hinge necessary for normal function, the structure and function of which is affected by FHC mutations. We are investigating the effects of these hotspot mutations using regulated in vitro motility (R-IVM) assays and transgenic mouse models. R-IVM data indicate that mutations Δ160E and E163R disrupt weak actomyosin binding interactions of the actomyosin crossbridge cycle. This is the first observation of FHC mutations in this region disrupting weak electrostatic interactions between the thin filament and myosin necessary for strong crossbridge formation. Δ160E and E163R transgenic mouse models show profound myofilament disarray. E163R animals show a less severe phenotype than Δ160E, in that a higher transgene dose of E163R tolerated and the sarcomeric ultrastructure is less severely affected. The structural and functional changes observed in vitro may contribute to the structural impairment seen in vivo by destabilizing myofilament structure and impairing dynamic functioning. These findings demonstrate a novel mechanism of disease for these hotspot mutations.

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Augmented-Reality Solution for Color Blindness

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An algorithm is demonstrated that can manipulate colors to enable perception by color-blind patients. A proposed implementation for patient use is via augmented-reality using a smartphone device, wherein a patient may hold up his or her phone facing the camera at a target view and be shown a perceivable image of the scene on the screen. Clinical testing can be performed using the Ishihara Color Test for color-blindness and it is shown that patients using the device can perceive the printed patterns on the plates. This technological approach enables rapid and low-cost distribution as well as potential personalized-medicine via custom calibration for individual patients' visions. Future forms may include head-mounted displays or active contact-lens implants that would enable passive visualization for the patient.

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The Role of Endogenous and Environmental AhR Ligands in Mammary Epithelial Cell Tumor Growth and Invasion

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Historically, AhR activation by environmental ligands was seen to facilitate mutations through CYP1 up-regulation. Our findings suggested that AhR activity drives cancer in the absence of environmental ligands. Our long-term goal is to identify endogenous ligands present in human mammary tumor cells, to determine how their production is controlled, and to assess how environmental AhR ligands alter this process. We hypothesize that metabolites produced by kynurenine (KYN) pathway of tryptophan metabolism drive AhR activity and enforce growth and/or invasion of malignant human mammary epithelial cells. As a corollary, we predict that environmental AhR ligands distort this signaling. We found that tryptophan metabolites kynurenine (KYN) and xanthurenic acid (XA), both detected in Hs578T cell lysates by LC/MS analysis, are potent AhR agonists. Hs578T cells highly express TDO, the rate-limiting enzyme in the kynurenine pathway. Knock-down of TDO using siRNA results in reduction of *Cyp1B1*, a transcriptional target of the AhR, as well as reduced expression of invasion-related genes *MMP1* and *9*, reduced cell migration in a wound healing assay and reversion of malignant phenotype in Matrigel. We also found that AhR activity contributes to transcriptional regulation of *TDO*. Our results support the hypothesis that AhR, hyper-activated by endogenous ligands produced via the KYN pathway, contributes to malignant phenotype in breast cancer. Moreover, our finding that the AhR transcriptionally regulates *TDO* expression leads us to propose a mechanism via which environmental AhR ligands lead to increased production of endogenous AhR ligands by up-regulating *TDO* and thereby enforce AhR-driven cell malignancy.

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L3MBTL2 Modulates DNA Repair in Breast Cancer

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DNA damaging agents are used extensively for cancer therapy. Though as little as one unrepaired double strand break (DSB) can be lethal to the cell, resistance and poor response rates are often seen; primarily because cells have developed a network of signaling pathways to sense and repair damaged DNA. Various proteins get recruited to the sites of DNA damage to facilitate cell cycle checkpoint activation and DNA repair. Here we demonstrate a critical role for Lethal (3) malignant brain tumor-like protein 2 (L3MBTL2), a novel polycomb protein, in selectively promoting error-free homologous-recombination (HR)-mediated DSB repair independent of cell cycle or transcription effects. Interestingly, the mechanism involves recruitment of key players in HR-mediated repair. Inhibition of L3MBTL2 lead to 4-fold increase in γH2AX foci, a marker of DNA double strand breaks (DSBs), in breast cancer cells. We further show that repair deficit due to downregulated L3MBTL2 can be exploited for therapy using Poly(ADP)-ribose polymerase (PARP) inhibitors which are selectively toxic towards HR-deficient tumors. Thus, this study improves our understanding of the molecular mechanisms that underlie chromosomal instability and elucidates biomarkers predictive of therapeutic response and resistance, which then may be used to profile and stratify patients in clinical trials. Additionally, by using targeted therapies that have already been shown to be well-tolerated, patient quality of life can be preserved.

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Online Information Seeking Behavior of Patients Newly Diagnosed with Urologic Cancers

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Introduction And Objectives: Numerous studies demonstrate patients are turning to the Internet in increasing numbers to acquire information regarding cancer diagnosis. Previous work in this field looked at self-reported Internet use (i.e., surveys, interviews) and objective website evaluation (i.e., readability, cultural sensitivity, and quality. Information on the Internet varies widely in readability, accuracy and scientific content. We hypothesize that patients with below average Wide Range Achievement Test (WRAT-4) scores and lower educational levels will be diverted to advertisements compared to those with above average WRAT-4 scores and higher educational levels. The goals of this study are to better understand how patients seek health information online and to determine if there are differences based on literacy and education levels. **Methods:** We performed a prospective, IRB approved study of patients with newly diagnosed urologic cancers. Patients diagnosed with cancer, underwent primary treatment and self-reported unfamiliarity with the Internet were excluded. After patient consent, they completed three activities: 1. Patients completed a demographic survey which also asked familiarity with the Internet. 2. Patients completed the WRAT-4 Blue Word Reading list; used as a proxy for literacy. 3. Patients spent five minutes on a self-directed Internet search about their cancer. Monitoring software was used to track all activity. All collected data was stored in REDcap, a secured Web database, for analysis. **Results:** Twenty-one patients were enrolled; nine with kidney cancer (42.9%) and twelve with prostate cancer (57.1%). Twenty patients used the Internet at home (95.2%) and eleven used it to find information about their cancer diagnosis (55%). Regarding literacy, mean standard WRAT-4 scores = 97.29, SD = 14.4 were below average. The frequently visited websites were advertisements (n=12), general health (i.e, WebMD, n=12), general cancer (i.e, cancer.gov, n=10), cancer-specific (n=8), and cancer treatment (n=7). Of all websites visited by those with only a high school education 40% were advertisements compared to 20% by those with at least a bachelor's degree. Patients with lower literacy frequently visited advertisements (81%). **Conclusions:** We demonstrated that patients with low literacy and education frequently visited advertisements compared to those with higher literacy and education when researching information about genitourinary malignancy. These findings should raise clinician awareness in order to guide patients to high quality websites and information on the Internet.

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Investigation of the Role of CD4+ T Cells and IL-10 in Immune-Mediated Neuroprotection

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A functioning immune system protects motoneuron survival after facial nerve transection. Further investigation revealed that CD4+ T cells and interleukin-10 (IL-10) are the key immune system components that prevent injury-induced motoneuron death. To better understand the mechanism of their action, this project will measure the effects of CD4+ T cells on gene expression profiles after injury, as well as determine the source and kinetics of IL-10 production. To study the effects of CD4+ T cells, wild-type (WT), immunodeficient recombinase activating gene-2 knockout (RAG-2 KO), and CD4+ T cell-reconstituted RAG-2 KO mice received a facial nerve transection. The mice are euthanized at specific time points, and the facial motor nucleus is laser-capture microdissected. RNA extraction will be performed on this tissue, and then the purified RNA will be reverse transcribed into complementary DNA for qPCR analysis. Preliminary findings reveal that a robust neuroregenerative response is present in all 3 experimental groups. However, there is significant dysregulation in the glial response to nerve transection in the RAG-2 KO mice compared to the WT and CD4+ T cell-reconstituted mice. These data suggest that CD4+ T cells regulate the glial response to injured motoneurons, and this factor likely contributes to motoneuron survival. To study the role of the anti-inflammatory cytokine IL-10, mice expressing an IL-10/green fluorescent protein (GFP) hybrid protein received a facial nerve transection, and immunohistochemistry was performed to identify colocalization of the IL-10/GFP with microglia or astrocyte cell markers. The results demonstrate that microglia are the cellular source of IL-10. *In situ* hybridization and qPCR of IL-10 mRNA expression will also be performed to confirm these findings. In summary, the current working hypothesis is that CD4+ T cells induce IL-10 production in microglia which, in turn, regulates glial activation and creates an anti-inflammatory environment that promotes motoneuron survival after injury.

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Insights Towards the Mitochondrial Pyruvate Carrier's Role in Cancer Metabolism

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Mitochondrial catabolism of carbon sources is an emerging hallmark of cancer. Otto Warburg first observed, through a series of ingenious and exceedingly simple experiments, that tumor cells shift their metabolism from mitochondrial oxidation of pyruvate to production of lactate, with a concomitant increase in glucose consumption. The nodal point at which this metabolic shift occurs is at pyruvate, the end product of glycolysis. Under normal aerobic conditions, pyruvate enters the mitochondria, where its oxidation to produce acetyl-CoA generates additional carbons to fuel the TCA cycle. In anaerobic conditions or in aerobic glycolysis, the vast majority of pyruvate is shunted into lactate, with its fermentation establishing a minimal redox balance for glycolysis to continue. The latter is known as the "Warburg Effect." Pyruvate metabolism can be modulated, at the very least, by no less than 4 mechanisms, and likely more, herein described as the "Mitochondrial Pyruvate Axis." All of which have been shown, in one context or another, to be "essential" to the metabolic programming of the cell. Three of these mechanisms are enzymes directly involved in the formation or destruction of pyruvate (pyruvate kinase (*PKM1, PKM2*); lactate dehydrogenase (*LDHA*); pyruvate dehydrogenase (*PDHA1*) and its regulatory kinases (*PDK1-4*) and phosphatases (*PDP1-2*); and the transporter responsible for pyruvate entry into mitochondria (*MPC1, MPC2*). Here we describe how manipulation of the Mitochondrial Pyruvate Carrier (MPC) in the context of colon cancer alters growth and metabolism of cells in culture and in xenografts. We also present new insights about the interplay between the MPC and other regulatory nodes of pyruvate metabolism in cancer and stem cells.

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Mini-Surgical Bladder Inoculation Reveals Male Susceptibility to Chronic Cystitis and Severe Pyelonephritis

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Urinary tract infections (UTIs) are primarily a disease of women; between the ages of 2 and 60 years, community-onset UTI occurs almost exclusively in females. However, UTI in male patients is neither nonexistent nor benign. Technical challenges in catheterizing male mice have heretofore precluded extensive study of male UTI and sex differences in these model hosts. We developed a novel mini-surgical bladder inoculation technique that yields reliable infection of the upper and lower urinary tract in male mice by uropathogenic *Escherichia coli* (UPEC), without peritonitis or systemic complications. Male and female C57BL/6 mice developed similar bladder bacterial titers and, by LacZ staining and confocal microscopy, there was no difference in the number or structure of intracellular bacterial communities. However, male C57BL/6 mice developed significantly higher UPEC titers in the kidney at 24 hours post-infection (hpi) and 2 weeks post-infection (wpi) compared to female mice. This sex difference was amplified in C3H/HeN mice, where males showed significantly higher renal bacterial titers at all time points examined, as well as a much higher (>95%) incidence of renal abscesses. In addition, bladder bacterial titers were higher in C3H/HeN males than females at 6 hpi and 2 wpi. Consistent with prior studies, a minority (24%) of C3H/HeN females proceeded to a high-titer, chronic active cystitis by 4 wpi; in contrast, 76% of C3H/HeN males demonstrated high bladder titers at 4 wpi. These males displayed persistent bacteriuria, had histological features consistent with the chronic cystitis previously reported in female hosts, and were indistinguishable from chronically infected females by confocal microscopy. Thus male sex in this model predisposes to more severe upper and lower UTI, including an increased incidence of chronic cystitis. Notably, these phenotypes were sharply abrogated in gonadectomized males, suggesting that male susceptibility to UTI is influenced by gonadal hormone exposure. Our mini-surgical model of UTI offers a highly tractable way to further interrogate sex differences in pathogenesis, host responses, and susceptibility to these common infections.

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Senescent Cell Clearance in High-Fat Fed Mice Improves Metabolic Phenotypes

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Cellular senescence is a basic aging mechanism that is a significant cause of tissue dysfunction with age. Cells undergo senescence, an essentially irreversible growth arrest, in response to significant stress including oxidative damage, metabolic stress, oncogene activation, or telomere shortening. Senescent cells have a unique phenotype, characterized by an enlarged cell size, accumulation of tumor suppressor proteins p16 and p21, and secretion of a host of cytokines, growth factors, and matrix remodeling proteins collectively known as the senescence-associated secretory phenotype, or SASP. The SASP may contribute to chronic, sterile inflammation that may play a key role in age-related disease. Senescent cell burden increases with age in many tissues and has been associated with disease states such as diabetes, primary sclerosing cholangitis, and dementia. In our laboratory, we have found that high fat feeding in mice causes increased senescence in adipose tissue including both subcutaneous and visceral depots. In these studies, we aimed to show that senescent cells accumulate in a mouse model of obesity, and to determine the impact of senescent cell clearance from high-fat fed mice on metabolic phenotypes associated with obesity. To achieve this, we used the novel p16-3MR mouse model, which contains a viral thymidine kinase gene driven by the p16 promoter that allows clearance of senescent cells expressing p16 upon Ganciclovir administration. We kept mice on a 60% fat diet for 10 weeks before administration of Ganciclovir in order to induce senescent cell formation. With Ganciclovir treatment, we confirmed reduced senescent cell abundance in adipose tissue via senescence-associated beta galactosidase staining as well as gene expression of senescence markers such as p21 and p16. Concurrent with senescent cell clearance, we saw improvements in metabolic phenotypes such as glucose and insulin tolerance and reduced microalbuminuria. Our results suggest that senescent cells play a role in metabolic and organ dysfunction associated with obesity, and that clearance of senescent cells, or ablation of the SASP, might prove to be an interesting therapeutic target in obesity and diabetes.

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Analysis of the Pathogenesis of Mouse Thymic Virus

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The Mouse Thymic Virus (MTV) is a naturally occurring mouse herpesvirus demonstrated to cause thymic necrosis and loss of CD4 + cells in infected BALB/c pups. Our studies focus on understanding the natural history of infection, thymic necrosis and resolution utilizing flow cytometry, genome copy number titering, histological analysis and electron microscopy of various organs at different time points after infection. We have demonstrated that depletion of thymic and splenic CD4 single positive cells begins five days post infection, with near complete loss of the population by day seven post infection. Double positive, CD4+CD8+ thymocytes, were decreased at day five and almost completely absent by day ten post infection. Recovery began approximately thirty five days post infection and completed by day forty five, with a seemingly complete reconstitution of both splenic and thymic CD4+ cells. Histological analysis is consistent with apoptosis and severe calcification peaking at twenty one days post infection. Viral genome copies are detected in the thymus throughout the course of infection, peaking at day seven, however, splenic viral copy number is near the lower limit of detection. These studies will prime our future work attempting to understand the mechanisms of pathogenesis and long-term consequences of MTV infection, with hopes of shedding light on generalizable principles of herpesvirus biology.

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Identification of SLE-Associated Risk Variants in the STAT1-STAT4 Locus

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Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease with debilitating inflammation that affects multiple organ systems. One of the first and most highly replicated genetic loci associated with SLE includes the *STAT1-STAT4* locus. Previous association studies assessed limited number of single nucleotide polymorphisms (SNPs) within the *STAT1-STAT4* locus, but none have identified the causal variant(s). In this study, we undertake an effort to identify all the SLE-associated common variants within the *STAT1-STAT4* locus. We genotyped 328 SNPs spanning the *STAT1-STAT4* locus (chromosome 2: 191.7MB - 192.2MB, Build37) in 13,581 subjects including individuals of European, Asian, African-American, and Ameri-Indian ancestries from the SLEGEN Immunochip Consortium. After imputing the genotped variants to a 1000 genomes composite reference panel, we applied a frequentist logistic regression in addition to a Bayesian strategy to identify the mechanisms leading to increased lupus risk. We identified a single genetic effect that accounts for the increased disease risk. We used Bayesian analysis to identify the thirteen genetic variants that account for 95% of the posterior probability in the region and make up the set of variants most likely to be causal. Critically, these findings were replicated in a large genetic data set from an independent cohort of 10,870 subjects from the Large Lupus Association Study 2 (LLAS2). In summary, we used two independent datasets to identify a set of variants that are most likely to be causal for the *STAT1-STAT4* association with increased lupus risk.

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Co-Development of the Infant Gut Microbiota and Immunoglobulin A Response

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Homeostatic interactions between the mucosal immune system and intestinal microbiota are critical for maintaining health. In infants, secretory immunoglobulin A (sIgA) responses develop in parallel with assembly of the gut microbiota. sIgA binds bacterial and food antigens, preventing them from directly interacting with the host by immune exclusion. The bacterial taxa targeted by sIgA in the developing gut, the environmental and genetic factors that shape sIgA responses, and the functional consequences of these immune-bacterial interactions remain poorly defined. We subjected 1670 fecal samples from 40 healthy mono- and dizygotic twin pairs living in the St Louis metropolitan area, collected monthly during the first 24 months of life, to 16S rRNA gene sequencing to determine the composition of the bacterial component of their microbiota. We selected a subset of 534 of these fecal samples, representing three-month intervals throughout the first two years of life, for fluorescence-activated cell sorting of IgA+ and IgA- fractions so that we could define the bacterial targets of mucosal immune responses within and across twin pairs, as a function of chronologic age, zygosity, and breast versus formula feeding practices. Additionally, we performed 16S rRNA sequencing and IgA profiling of 97 fecal samples collected from the mothers of these twins near the time of parturition and during the first 36 postnatal months. Using Random Forests, a machine learning algorithm, and the 16S rRNA datasets, we have generated a sparse 24-taxon model composed of age-indicative bacterial strains that together describe assembly of the gut microbiota in this healthy population. Using this approach, we also constructed another model that describes the maturation of mucosal IgA responses. Finally, we have colonized gnotobiotic mice with fecal microbiota obtained from these twin pairs as part of our effort to characterize how microbiota assembly/maturation and mucosal IgA responses are impacted by various types of foods commonly consumed by infants in the USA as they transition to solid foods.

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Development and Evaluation of Silk-Elastinlike Protein Polymers as Injectable Chemoembolic Systems for the Treatment Hepatocellular Carcinoma

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Introduction: Hepatocellular carcinoma (HCC) annually affects over 700,000 patients worldwide. Majority of patients are ineligible for surgery and undergo treatments like transarterial chemoembolization (TACE) as palliative care or bridge to transplant. This treatment involves accessing the tumor vascular supply via catheter and injecting a combination of chemotherapeutics and an embolizing agent. We are investigating recombinant silk-elastinlike protein polymers (SELP) as an alternate embolic material with capacity to serve as a drug carrier. SELPs are genetically engineered polymers that when formulated as an aqueous solution will transition into a gel network at physiological temperature. This novel embolic can permeate tumor vessels down to capillary levels and control drug release after gelling. The goal of this work developing a SELP formulation that can be mixed with a combination of sorafenib encapsulated poly(lactic-co-glycolic acid) (PLGA) nanoparticles and doxorubicin for the treatment of HCC. **Methods:** Several SELP constructs were investigated to determine a candidate meeting described specifications. Constructs 47K and 815K, where the first number refers to the number of silk units (4 or 8), and the second, the number of elastin with one lysine modified unit (7K, 15K) in each monomer, were formulated at different concentrations by weight using lyophilized protein made into an aqueous suspension followed by shear processing. Physical characteristics were measured using rheological techniques. Viscosity in the temperature range of 18-23°C was specified to be <150cP to achieve injectability through a microcatheter of internal diameter 2.8 F. Gel formation upon injection into a 37°C system was specified to complete in <5 minutes. Final gel strength was specified to be > $1x10^5$ Pascal. A finalized formulation, sheared 12% w/w 815K was identified as a candidate, which underwent *in vitro* and *in vivo* feasibility testing in rabbits. Next stage, a drug delivery component was added to develop a complete chemoembolic system. PLGA nanoparticles were synthesized encapsulating anti-angiogenic sorafenib to be incorporated into the SELP matrix. The PLGA provides a level of controlled release. Liposomal formulations of doxorubicin were also tested for release profiles out of the 815K gel matrix. **Results:** The sheared 12% w/w 815K showed selective occlusive ability within branches of the hepatic artery. The biomaterial gelled and remained lodged within the vasculature and did not drain into the venous and pulmonary circulations. PLGA nanoparticles encapsulating sorafenib were successfully synthesized. Individual and co-release studies of these drug systems from the SELP matrix are underway to determine effectiveness of SELP as a chemoembolic for the treatment of hepatocellular carcinoma.

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Recurrent Copy Number Variants and Bicuspid Aortic Valve are Enriched in Early Onset Thoracic Aortic Aneurysms and Dissections

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Background: Thoracic Aortic Aneurysms and Dissections (TAAD) are a major cause of death in the United States. The spectrum of TAAD ranges from congenital disorders such as Marfan syndrome to sporadic isolated disease of unknown cause. We hypothesized that genomic copy number variants (CNVs) contribute causally to early onset TAAD (ETAAD). **Methods:** We conducted a genome-wide SNP array analysis of ETAAD patients of European descent who were enrolled in the National Registry of Genetically Triggered Thoracic Aortic Aneurysms and Cardiovascular Conditions (GenTAC). Genotyping was performed on Illumina Omni-Express beadchips, using PennCNV, Nexus Copy Number and CNVPartition for CNV detection. 110 ETAAD patients (100% Caucasian, 24% female, average age 18, 60% bicuspid aortic valves) were compared to 3450 dbGAP controls without a history of vascular disease using association tests for rare CNVs as implemented in PLINK. Thresholds were set at a minimum of six consecutive SNPs for CNVs that were identified by at least two algorithms and intersect with known genes. For comparison, 805 elderly, sporadic TAAD patients (STAAD group) and 88 probands from families with at least two affected relatives (FTAAD group) from our institutions were screened for additional CNVs at these loci with SNP arrays. Pathway and functional analyses of rare CNVs were performed using Ingenuity Pathways Analysis software. **Results:** We identified 27 recurrent CNVs in the ETAAD, FTAAD and STAAD groups that were absent or extremely rare (frequency <0.1%) in controls. Nine rare CNVs that were either very large (>1 Mb) or shared by ETAAD and STAAD or FTAAD patients were also identified. Four rare CNVs involved genes that cause arterial aneurysms when mutated. The largest and most prevalent of the recurrent CNVs were at Xq28 (four duplications and two deletions) and 17q25.1 (three duplications). The frequency, size and genic content of CNVs were all significantly increased in ETAAD cases compared with controls ($P<0.001$). The percentage of individuals harboring rare CNVs was significantly greater in the ETAAD cohort (33%) than in the FTAAD (23%) or STAAD (15%) cohorts. **Conclusions:** The prevalence of BAV is remarkably enriched among ETAAD cases (60%) compared with the general population (1-2%). We identified multiple loci affected by rare CNVs in one-third of ETAAD patients, demonstrating the genetic heterogeneity of TAAD and implicating alterations of candidate genes at these loci in the pathogenesis ETAAD and/or BAV. BAV-associated aortopathy may interact with these genetic variants to drive early aneurysm formation.

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Toxicity Level of Standardized Mangosteen (*Garcinia mangostana*) Pericarp Ethanolic Extract on Zebrafish (*Danio rerio*) Embryos

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Garcinia mangostana (Mangosteen) has a wide range of benefits, perhaps most result from xanthone phytoceuticals/antioxidants. Garcinia mangostana perceived in Indonesia as the king of the antioxidant, however we already know that every potent medication has a limitation on use and toxic level. The toxic level perceived to be the effect of other phytoceutical compound, named mangostin, that responsible for the mortality and morbidity mechanism of the animal model. Reported effect of the overconsuming mangosteen pericarp extract such as metabolic disorders, hemolysis, lymphocytosis, and decrease of liver and kidney masses. The purpose of this study is to know the acute toxicity level in terms of mortality rate, lethal concentration, and the teratogenic effect of mangosteen pericarp ethanolic extract toward zebrafish embryos. Experimental study used zebrafish embryos placed in 6-Well, each well contained 30 embryos divided in 4 groups, there were three groups were given standardized mangosteen pericarp ethanolic extract (1250, 1000, 750 µg/mL) and one control group was given with physiological embryonic medium which observed every 24 hours for 3 days and the study repeated three times. Zebrafish (*Danio* rerio) has 70% autologous toward human, therefore zebrafish also being used for toxicity test toward natural compound. The extract was given on 2 hpf (hour post-fertilization). The results show that on 1250 µg/mL concentration of mangosteen pericarp ethanolic extract, mortality rate reach 100% at three times repetition of the study since 24 hpf. At 1000 µg/mL concentration, the mortality rates for the first, second, and third repetition are 71%, 70%, and 63% consecutively. At 750 µg/mL concentration, the mortality rates for the first, second, and third repetition are 13%, 33%, and 23% consecutively. We were using SPSS Ver.22 program for Probit Analysis, the lethal concentration for half population (LC50) of mangosteen pericarp ethanolic extract was reached at 716,651 µg/mL. Defects were found at 750 µg/mL concentration at 72 hpf observation, in the forms of curved-shape body, broken or enlarged pericardium, and undetected heart rate because the pericardium cavity was murky although pigment has been formed in several skin parts of embryos.

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Using Targeted Next Generation Sequencing (NGS) to Identify Putative Mutations in Genes Associated with GnRH Development and Hypogonadotropic Hypogonadism

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Coordination of the hypothalamic-pituitary-gonadal axis, which is governed by pulsatile release of gonadotropin-releasing hormone (GnRH), is essential for normal reproductive competence. GnRH is secreted from hypothalamic neurons that have migrated along with olfactory neurons from the nasal region to the arcuate nucleus during embryologic development. Disruption of GnRH neuron migration and/or interference with GnRH secretion results in hypogonadotropic hypogonadism, which may be normosmic (nHH) or anosmic/hyposmic Kallmann syndrome (KS). These patients with nHH/KS have in common a clinical presentation of absent puberty, low or inappropriately normal gonadotropins, and deficient sex steroids. The molecular basis of nHH/KS has been ascertained in 30-40% of patients, and may be attributed to mutations in one or more of 18 genes. The genetic basis of nHH/KS has been elucidated by a variety of techniques including candidate gene approaches, linkage analysis, and positional cloning using chromosomal deletions and/or rearrangements. However, since a majority of the molecular etiologies for nHH/KS have not yet been determined, next generation DNA sequencing represents a promising methodology for new gene discovery. Whole exome sequencing and whole genome sequencing have been used, but bioinformatic analysis may be challenging because of the large number of DNA sequence variants identified. We therefore employed a targeted sequencing approach. Utilizing in silico databases, pathway analysis, and relevant literature, approximately 285 genes were selected to study in 48 nHH/KS patients without mutations in known genes. Candidate genes were arbitrarily categorized as being involved in hypothalamic development and patterning; olfactory development; GnRH neuron development, migration and signaling; and pituitary regulation of gonadotropin secretion. Known causative genes and additional candidates, as identified by position in nHH/KS patients with balanced chromosomal rearrangements, were also included. SureDesign Software (Agilent Technologies) was used to design probes for the sequence of the 285 candidate genes. Coding exons were captured using the Agilent HaloPlex custom capture kit and DNA sequenced by Illumina Hiseq2000, which provided paired ends reads (2x 90) achieving 30-175 fold coverage of genomic sequence. DNA sequencing is currently being performed, but preliminary DNA sequence variants are being confirmed and studied in family members and controls. Targeted next generation sequencing of relevant developmental genes may provide an additional useful approach to identify new genes involved in human pubertal development.

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An fMRI Investigation of the Neural Correlates of the Restorative Effect of Nature

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While the positive effects on cognition and well-being demonstrated by nature and greenery are well known, the neural correlates of these effects are underexplored. In this study, ten participants observed images from each of three different categories: Nature images, positive non-nature images, or neutral objects while they underwent fMRI (BOLD) scanning. Nature images elicited significantly more bilateral activation in the frontal, sensorimotor, and cerebellar regions relative to neutral objects, which may be associated with imagined movement through space and reflect high level engagement of visuospatial attention. Nature images also produced significantly more activation in left-lateralized brain regions, many of which belong to the default mode network (DMN)Relative to positive non-nature images, which plays a role in mediating self-awareness (Raichle et al. 2001). These results provide support for Kaplan's Attention Restoration Theory as it pertains to nature environments, particularly as our findings suggests neural correlates for the constructs of "soft fascination" and "reflection" via the DMN (Kaplan & Berman, 2010).

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Empirical Lambda Distribution Aids Interpretation of False-Positive Rates from a Product-Term Model of Gene-Gene Interaction Applied to Complex Traits in Humans

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In genome-wide association studies, measurement of false-positive (type I error) rates has been an important quality-control procedure allowing investigators to conclude that p-values are not systematically biased high or low due to artifacts such as population substructure, and are therefore interpretable. Similar procedures will be necessary as large-scale genotype-phenotype mapping studies of complex traits expand to gene-gene and gene-environment interactions. A common method of testing for interaction is a least-squares regression analysis using two variables and their product (representing the interaction) as predictors. After observing much variability in false-positive rates when applying this model to quantitative traits in the NHLBI Family Heart Study, we sought to further characterize this variability and determine how to interpret p-values in its presence. We performed 81 SNP-by-genome interaction scans on a sample of 1053 unrelated European-Americans from the Family Heart Study, and additional scans on each of 3 progressively larger simulated datasets which did not contain population substructure or SNP effects. This allowed us to construct empirical distributions of the product-term genomic inflation factor (lambda) value and to observe the effects of sample size, minor allele frequency, and heteroskedasticity on these distributions. Because the variation was strongly related to heteroskedasticity at low sample size, and because heteroskedasticity-consistent standard errors have been suggested by a few authors as a way of correcting lambda inflation in gene-environment interaction scans, we also tested two types of these standard errors (HC0 and HC3) on our data. We explain the lambda variation as a result of non-independence of interaction-term test statistics within each scan, coupled with minor biases in test statistics due to a failure to reach asymptotic properties. While heteroskedasticity-consistent standard errors narrowed the range of lambda, with HC3 outperforming HC0, they came with the trade-off of creating p-value outliers that appeared to be related to sparseness of two-locus genotype classes. We propose that under certain conditions, one way to interpret interaction-term lambda values is by using least-squares standard errors but comparing lambda to an empirical lambda distribution generated from a simulated dataset without SNP effects or population substructure. Based on our results these distributions are broader than those observed in main-effect-only GWAS. We further conclude that if heteroskedasticity-consistent standard errors are used to improve lambda values, HC3 is preferable to HC0 and both come with caveats, notably that the choice of minor allele frequency threshold becomes critical in order to avoid the creation of new false-positives.

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EHD1: A Regulator of Membrane and Microtubule Dynamics During Cytokinesis

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The maintenance of genomic integrity is paramount to tissue homeostasis in the human body. Indeed, the failure to complete cytokinesis, the last stage of the mitosis, creates tetraploid cells and genomic instability. Strong evidence supports a role for genomic instability as an etiologic event in pancreatic, colorectal and lung tumors. Our previous work demonstrated that the endocytic regulatory protein EHD1 (Eps15 homology domain containing protein1) is required for cytokinesis, as siRNA-mediated depletion of EHD1 caused tetraploidy. The goal of our current work is to elucidate the mechanism by which EHD1 regulates cytokinesis in human cells. Using a combination of fixed and live cell confocal microscopy and super-resolution Structured Illumination Microscopy (srSIM), we find that EHD1-depletion disrupts central spindle organization. In control cells, the plus ends of microtubules terminate at the equator or center of the central spindle. However, in EHD1-depleted cells, plus ends fail to terminate at the equator. Subsequently, the organization of the molecular machinery that controls the furrowing process is mis-localized and not focused at the equator, leading to less robust and unstable furrows. Mechanistically, we provide evidence that EHD1 is involved in the localization of several key central spindle regulatory proteins including the mitotic kinase Aurora B and the microtubule motor KIF4a. Both Aurora B and KIF4a translocate from chromosomes to the central spindle and control central spindle plus end dynamics. In EHD1-depleted cells, Aurora B and KIF4a fall to localize to the central spindle equator and

remain near the spindle poles. In total, our data suggests a novel role of the endocytic regulatory protein EHD1 in mediating microtubule dynamics and plus end trafficking during cytokinesis.

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YAP Promotes Biliary Oncogenesis by Upregulation of Fibroblast Growth Factor Receptors

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Background and Aims: Cholangiocarcinoma (CCA) is a lethal malignancy originating from the biliary tract with a dismal prognosis and limited treatment options. Fibroblast growth factor receptor (FGFR) fusion genes have recently been described as key driver mutations in CCA. This observation suggests FGFR upregulation by alternative mechanisms may participate in CCA genesis and progression. In addition, activation of Yes-associated protein (YAP), a transcriptional co-activator in the Hippo signaling pathway signaling, has also been identified in human CCA. Our aim was to examine cross-talk between these pathways to identify therapeutic strategies for these cancers. **Methods**: Human cholangiocarcinoma specimens were examined by immunohistochemistry, western blot, and qRT-PCR. CCA cell lines were used for *in vitro* studies. *In vivo* studies were conducted in CL57BL/6 mice (N=20) which underwent biliary transduction of constitutively active AKT and YAP, a novel model of murine carcinogenesis recently developed by us. From weeks 6-8, mice received either vehicle or the FGFR-specific inhibitor, BGJ398, via daily oral gavage. All mice were sacrificed at the end of week 8 and examined for the presence of tumors and tumor burden. **Results:** YAP nuclear localization, indicating YAP activation, was identified in ≥ 50% of resected human CCA specimens. YAP nuclear localization and upregulation of YAP transcriptional targets was observed in human CCA cell lines but not normal human cholangiocytes. Human CCA specimens and cell lines also had enhanced expression of FGFR 1, 2, and 4, and their respective ligand FGF2; expression of the receptors was decreased in cell lines stably transfected with short hairpin RNA-mediated knockdown of YAP. In addition, tumor tissue from a YAP-driven murine model of CCA demonstrated increased expression of FGFR 1, 2, and 4. The promoter region of all FGFR isoforms contained a TBX5:YAP binding sequence. Chromatin immunoprecipitation assay was utilized to confirm YAP binding to the cognate DNA sequence. The pan-FGFR inhibitor, BGJ398, inhibited YAP nuclear localization and downregulated YAP target genes such as SOX4 and CTGF in CCA cell lines. Moreover, BGJ398 induced apoptosis likely by decreasing cellular levels of the anti-apoptotic protein Mcl-1. BGJ398 reduced tumor burden in a YAP-driven genetic murine model of CCA, likely via increased apoptosis. **In Conclusion**, YAP is a critical oncogene in CCA and promotes biliary carcinogenesis, in part, by upregulation of FGFR. The FGFR specific inhibitor BGJ398 significantly reduces tumor growth and progression in a YAP-driven murine model of CCA. Thus, inhibition of FGFR represents a promising therapeutic approach in a subset of human CCA.

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Targeting Epithelial to Mesenchymal Transition Through CXCR4 Blockade

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Multiple osteolytic lesions are one of the main clinical features of patients with multiple myeloma (MM), thus suggesting the ability of clonal plasma cells to disseminate from bone to bone. The bone marrow (BM) homing process of MM cells is supported by the CXCR4/CXCL12 axis activation. Nevertheless, the role of CXCR4 in mediating MM cell bone metastasis, and the role of CXCR4-targeted therapy in inhibiting MM cell metastasis from bone-to-bone has not been previously reported. We categorized BM-MM-derived CD138+ cells (GSE24080; n=577) according to their CXCR4 mRNA expression; and identified an EMT-related pathway to be significantly activated in high-CXCR4- versus low-CXCR4-BM-MM CD138+ cells (FDR: 0.19; *P* 0.02), as shown by using GSEA and Atlas of Cancer Signaling Networks. We next performed CXCR4-gain-of-function studies and demonstrated that CXCR4-overexpression led to changes in actin cytoskeleton, with protrusion of cell pseudopodia, sustained by modulation of EMT-related markers (Slug/Snail/Twist up-regulation; E-cadherin down-regulation), compared to control cells. These findings were recapitulated using an in vivo model of MM cell bone-to-bone metastasis: CXCR4-overexpressing MM cells presented with higher bone-to-bone metastasization compared to control cells, as confirmed on harvested host femurs (IHC; flow cytometry). Lower expression of human (h)-E-cadherin, and higher expression of h-Twist/h-Snail/h-Slug was confirmed within the BM of the host femurs. We next tested the novel monoclonal antibody, anti-CXCR4 (BMS-936564). BMS-936564 exerted an anti-MM activity in situ, within the s.q. implanted bones; and also reduced MM cell dissemination from the implanted bone to the host bone. We examined whether BMS-936564 might have modulated EMT-related genes in the MM cells metastasized to the host bones, and found enhanced mRNA expression of human (h)-E-cadherin, and reduced expression of h-Twist/h-Snail/h-Slug and h-CXCR4 in the BM cells harvested from the host bones of BMS-936564-treated mice. Similar findings were confirmed in a breast cancer in vivo model (MDA-MB-231 intra-cardiac injected), where BMS-936564 led to prolonged survival. CXCR4-silenced MM cells presented with inhibited BM homing (intra-vital confocal microscopy); inhibited MM tumor growth in vivo (BLI); and prolonged survival compared to control mice injected with scramble probe-infected cells. Moreover, BMS-936564 led to inhibition of MM tumor growth in vivo, using MM.1S- and RPMI.8226- xenografr s.q.models; with a synergistic effect when used in combination with lenalidomide or bortezomib. Our study supports the pro-metastatic role of CXCR4, due to its effect in mediating EMT in both MM and breast cancer in vivo models, thus suggesting the potential role for using CXCR4-neutralizing agents in order to delay or prevent tumor cell metastasis to the bone.

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C-kit Signals Differentially Regulate ILC2 Accumulation and Susceptibility to CNS Demyelination in Male and Female SJL Mice

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Females are more susceptible to autoimmune diseases, including multiple sclerosis (MS), a T-cell mediated, demyelinating disease of the central nervous system. Although genetic, hormonal, and immune differences have been implicated; the specific mechanisms that underlie this female predominance remain unclear. The SJL mouse model of MS, experimental autoimmune encephalomyelitis (EAE), recapitulates many of the features of the human disease; including the relapsing-remitting course and sex dimorphism. When immunized with the myelin peptide $PLP_{139-151}$, female SJL mice develop characteristic episodes of severe clinical disability interspersed with pronounced clinical remissions. In contrast, males exhibit only mild clinical symptoms, if any. We previously reported that c-kit, the receptor for stem cell factor, regulates disease susceptibility in SJL females through its effects on mast cell survival. c-kit mutant (*Kit*$^{W/Wv}$) SJL female mice are mast cell deficient and exhibit significantly less severe disease than their wild type (WT) counterparts. Mast cell reconstitution restores WT-like disease susceptibility. Despite equivalent peripheral myelin-specific T cell responses, immune cell influx into the CNS is compromised in *Kit*$^{W/Wv}$ female mice, indicating that mast cells control inflammatory cell access to the CNS in females. In contrast, male SJL-*Kit*$^{W/Wv}$ mice develop significantly more severe EAE than their WT littermates. This difference in disease severity corresponds to a more robust peripheral $CD4^+$ T cell cytokine response during the preclinical phase of disease and is independent of mast cells, intrinsic T cell function, and serum testosterone concentrations. Rather, reduced accumulation of type 2 innate lymphoid cells (ILC2s) corresponds to disease susceptibility. ILC2s are a recently described cell population that maintains c-kit expression in maturity and simultaneously promotes a Th2 while limiting a Th1/17 response. ILC2s are increased in the lymph nodes and central nervous system of EAE-resistant WT males compared to EAE-susceptible *Kit*$^{W/Wv}$ male and WT female mice. We propose that a deficiency in the ILC2 population removes an attenuating influence on the autoreactive T cell response and therefore, increases disease susceptibility in both *Kit*$^{W/Wv}$ male and WT female mice. Thus, c-kit exerts distinct disease-modifying effects in male and female mice, most likely by affecting ILC2 function.

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Time-Course Analysis of CLDN-4 Expression After Treatment of Astrocytes with IL-1β

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Currently, multiple sclerosis is an incurable, inflammatory demyelinating disease of the CNS white matter that afflicts over 2.5 million people with a large number of symptoms across the world. Breakdown of the BBB is a central pathogenic step in the initiation and progression of auto-inflammatory demyelinating disease. Local inflammatory cytokines secreted during this disease, in particular IL-1β, stimulate resident astrocytes to be reactive. These secrete VEGF-A which induces a down-regulation of endothelial tight junction molecules, like Claudin-5 thereby weakening BBB and allowing for an ingress of damaging inflammatory leukocytes into the CNS parenchyma. Interestingly the reactive astrocytes also express tight junction proteins such as claudin4 (CLDN-4) which seals off the glia limitans, thus controlling inflammatory cell access into the brain. The glia limitans is a thin barrier of the astrocytes that exists between the CNS parenchyma and the perivascular space, which surrounds the blood vessels of the brain. However, the exact mechanism of how CLDN-4 is expressed, how it is regulated and what exactly is its function within this ecosystem is still unclear. In this study, CLDN-4 expression was analyzed across various time intervals after treatment of the astrocytes by inflammatory IL-1β. After initial protein immunoblot analysis, we were not able to determine a consistently clear analysis on the regulation or nature of CLDN-4 expression levels. By determining the levels of CLDN-4 expression we can better understand the complex cellular mechanisms and pathways behind this inflammatory attack which will help us develop strategies to minimize clinical disability from early inflammatory lesions of the brain and the spinal cord.

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The Role of miR-122 in the Pathogenesis of Alcoholic Liver Disease

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Purpose/Background: The progression of alcoholic liver disease (ALD) is multifactorial, involving both metabolic and immunological dysfunctions. MiR-122 has been shown to regulate essential functions in hepatic lipid metabolism, mitochondrial function, cell death pathways, fibrosis and carcinogenesis – major elements in ALD. While recent studies have demonstrated the therapeutic benefits of miR-122 inhibition in HCV infection, we have observed the reduction of miR-122 expression in the livers of alcohol-fed mice. Given the highly conserved role of this unique miRNA in hepatic homeostasis, we hypothesized that the loss of miR-122 contributes to ALD progression and may be a therapeutic target. In this study, our goals were two-fold. First, we aimed to assess the effect of miR-122 inhibition on steatosis, inflammation, and fibrosis in ALD. Second, we sought to therapeutically restore miR-122 in the livers of alcohol-fed mice to alleviate liver injury. **Methods:** Wild-type 6-8 week old, female C57Bl/6 mice were injected intravenously with scAAV8 viral particles containing anti-miR-122 TuD (TuD), or scrambled vector (scr). After 14 days, mice were started on a Lieber-DeCarli (LDC) alcohol diet or calorie-matched control diet (PF) for four weeks. Additionally, WT mice were treated with scAAV8 pri-miR-122 (OX) on day 7 of the LDC diet. **Results:** Knockdown of miR-122 in the liver resulted in substantial increases in ALT and weight loss in both PF and Et groups compared to their respective Scr-treated controls. Cytokine analysis and histologic evaluation (H&E) demonstrated significant increases of steatosis and inflammatory cell infiltration in TuD+PF and TuD+et mice. Sirius Red staining revealed induction of early fibrosis in TuD+et mice compared to controls. This was further corroborated by increased expression of procollagen 1-α and α-smooth muscle actin in both TuD+PF and TuD+Et groups compared to their respective Scr controls. Finally, miR-122 overexpression by treatment with scAAV8 OX in WT, alcohol-fed mice resulted in a significant decrease in serum ALT suggesting a protective role for restoration of miR-122 levels in ALD. **Conclusion:** Our results suggest that inhibition of miR-122 by alcohol is a key element of the pathogenesis of ALD. miR-122 inhibition alone mimics the steatosis, inflammatory cell invasion and activation as well as early fibrosis seen in chronic-alcohol treatment. Reconstitution of miR-122 expression in the livers of alcohol-fed mice results in a significant reduction of alcohol-induced liver damage. Our findings demonstrate the ability of miR-122 expression to modulate liver injury and its potential as a treatment for ALD.

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Cell Size-Specific Intracellular Delivery

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Among the many methods of intracellular delivery, cell size-selective delivery is particularly applicable to cancer research and therapeutics where tumor cells tend to be larger than blood cells and selective manipulation of a single cell-type while minimally affecting the other cells in a heterogeneous mixture is important. In this study, cell size-selective delivery is achieved using a novel microfluidic device with 75 parallel channels through which cells are pushed under nitrogen pressure. The cells undergo deformation as they transit through the channels, which results in temporary disruption of the cell membrane to facilitate delivery of material into the cytoplasm. For each cell size, there is a specific channel width for which optimal cell viability and fluorophore delivery is achieved, with smaller cells requiring narrower channels. When two cells of different sizes are mixed in solution, the channel width for optimal cell viability and fluorophore delivery for each cell type remains the same, and larger cells can achieve fluorophore delivery at a significantly higher percentage than smaller cells at the former's optimal channel width. One possible application for this technology is tagging circulating tumor cells, and we have been able to selectively deliver fluorophores into tumor cells when spiked into whole human blood with 91% specificity. We were also able to isolate pancreatic tumors cells from a patient's blood sample that matched the genotype of the patient's primary pancreatic tumor. Intracellular delivery of materials has become increasingly important as we delve deeper into understanding cellular processes and developing targeted therapies, and with this device, selective delivery can be achieved in a vector-free environment and without dependence on cell-surface receptors.

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Development of Nanoformulated Copper/Zinc Superoxide Dismutase as an Alternative Therapeutic Strategy for Angiotensin II-Dependent Hypertension

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Excessive generation of superoxide ($O_2^{\bullet-}$) has been extensively implicated as a signaling molecule in cardiovascular pathologies, including hypertension. As a major risk factor for myocardial infarction, stroke, and heart failure, the morbidity and mortality associated with hypertension is a worldwide epidemic. Although there are several standard therapies that effectively lower blood pressure, many hypertensive patients have uncontrolled blood pressure despite taking available medications. Thus, there is a necessity to develop new pharmacotherapies that target novel molecular effectors (e.g. $O_2^{\bullet-}$) that have been implicated to be integral in the pathogenesis of hypertension. To overcome the failed therapeutic impact of currently available antioxidants in cardiovascular disease, we developed a nanomedicine-based delivery system for the $O_2^{\bullet-}$ scavenging enzyme, copper/zinc superoxide dismutase (CuZnSOD), in which CuZnSOD protein is electrostatically bound to poly-L-lysine (PLL_{50})-polyethylene glycol (PEG) block co-polymer to form CuZnSOD nanozyme. Different formulations of CuZnSOD nanozyme are covalently stabilized by either reducible or non-reducible crosslinked bonds between the PLL_{50}-PEG polymers. Herein, we tested the hypothesis that PLL_{50}-PEG CuZnSOD nanozyme delivers active CuZnSOD protein to neurons and decreases blood pressure in a model of AngII-dependent hypertension. Utilizing electron paramagnetic resonance (EPR) spectroscopy, we determined that nanozymes retain full SOD enzymatic activity in a cell-free environment. Furthermore, non-reducible crosslinked nanozyme delivers active CuZnSOD protein, as analyzed by western blot and CuZnSOD activity assays, to central neurons in culture (CATH.a neurons) without inducing significant neuronal toxicity. *In vivo* studies conducted in AngII-mediated hypertensive adult male C57BL/6 mice demonstrate that the non-reducible crosslinked nanozyme significantly attenuates blood pressure following a single bolus intracerebroventricular injection (mean arterial pressure (MAP) significantly decreased within 24 hours and for up to 7 days, $p<0.05$). Furthermore, there was a significant difference in MAP between mice intravenously injected with non-reducible crosslinked nanozyme or saline for the duration of 10 days post-injection ($p<0.05$). While these physiologic data are promising, the biological distribution of our nanozymes implicate the proximal tubules in the kidney cortex as a primary target for CuZnSOD nanozyme following IV administration. Collectively, these studies support the further development of PLL_{50}-PEG CuZnSOD nanozyme as an antioxidant-based therapeutic option for the improved treatment of hypertension. Moreover, the therapeutic impact of CuZnSOD nanozyme may be investigated in additional pathologies in which there are excessive levels of $O_2^{\bullet-}$ present in the kidney.

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Loss of the Mitochondrial Pyruvate Carrier (MPC) is a Novel Mediator of the Warburg Effect

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Limiting the access of pyruvate to the mitochondria is perhaps the simplest and most efficient way to enact aerobic glycolysis also known as the Warburg effect. Simply blocking the entry of pyruvate to the mitochondria will result in aerobic glycolysis without any other changes in glycolytic or oxidative pathways. Supporting this, we have shown that glycolytic cancers have lower levels of *MPC1* and *MPC2* genes, which form the mediator of mitochondrial pyruvate entry now known as the MPC. In addition, low levels of the MPC correlate strongly with poor prognosis, more so than other commonly observed metabolic alterations in cancer. *MPC1* also lies within the region most commonly deleted across all cancers. Following the observations that the MPC is reduced in many cancers, we interrogated its role via stable re-expression of *MPC1* and *MPC2* in colon cancer cell lines. Compared to control, cells expressing MPC displayed reduced glycolysis and increased mitochondrial metabolism of pyruvate at baseline and when maximally stimulated. While adherent growth, viability, and measures of apoptosis were unaltered, we observed a profound defect in the ability of cells expressing the MPC to form colonies in soft agar as well as tumors in xenografts (Soft Agar: 80-90% reduction, $p < 0.01$; Xenograft: 30-60% reduction, $p <0.0001$). The addition of the MPC inhibitor UK5099 was able to rescue this growth defect in spheroids, showing that it is the entry of pyruvate into the mitochondria that is deleterious to growth in these conditions. As the low attachment spheroid and soft agar colony formation assays are commonly used to interrogate stem-like properties of cancer we next assessed common markers of cancer stem cells. Compared to control cell lines, those expressing MPC have significantly reduced protein expression of common stem cell markers including LGR5, Nanog, ALDHA1, and Lin28A by western blot. These changes were consistent across normally growing adherent cells as well as in the tumors formed from those cells in xenografts. Cell surface expression of CD44, a commonly used marker of cancer stem cells was significantly reduced, as was the population of cells with high activity of the intracellular enzyme ALDHA. Most strikingly, this defect was traced to the cancer stem cell population. The capacity of these cancer stem cells to form spheroids was reduced with MPC re-expression while the non-stem population displayed no difference. This work implicates the MPC as a novel mediator of the Warburg effect and shows its suppression is necessary to maintain the cancer initiating cell population.

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Characterization of Anoikis-Resistance in Human Osteosarcoma Reveals Novel Signaling Changes and Sensitivity to Targeted Epigenetic Therapies Predicted by Expression Profiling

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Background: Osteosarcoma (OS) is the most common type of solid bone cancer, with latent metastasis being a typical mode of disease progression and a major contributor to poor prognosis. Anchorage-independent (AI) growth is an essential feature of malignancy and metastatic progression. The cancer cells must resist anoikis, i.e. programmed cell death in the absence of attachment to extracellular matrix, for this to occur. Here we sought to characterize the functional consequences of anoikis-resistance in human OS using a model of AI cell growth, with the ultimate goal of identifying novel signaling changes to target OS subpopulations principally responsible for micrometastatic dissemination. **Methods/Results:** Human OS cells were cultured in adherent or AI conditions using ultra-low attachment plates. AI-grown cells exhibited decreased proliferation compared to their adherent counterparts *in-vitro* as determined by decreased cell doublings per day as well as decreased Ki67 protein via western blot, while AI growth promoted resistance to both doxorubicin and cisplatin. AI OS cells were equally tumorigenic as adherent cells *in-vivo* in orthotopic intratibial xenografts, but maintained their slower proliferation rate. Gene expression profiling of OS patient-derived cell isolates grown in AI or adherent conditions with principal component and pathway analysis revealed consistent differences in the gene expression of AI and adherent OS cells. Top ranked pathways centered around c-myc and β-catenin, while shortest pathway analysis revealed significant convergence on Runx2, Akt, and class I histone deacetylases (HDACs). Expression of 19 genes passed false discovery as being significantly altered in AI cells compared to adherent cells, which included Runx2 and CTGF, a target gene of the Hippo signaling mediator YAP. We noted upregulation of YAP, Runx2, and both active and total β-catenin in AI cells via western blot. Finally, we validate the use of two FDA-approved epigenetic therapies *in-vitro*, showing that both the pan-HDAC inhibitor vorinostat and the DNA methyltransferase inhibitor 5-azacytidine reduce AI cell growth, while 5-azacytidine sensitizes anoikis-resistant cells to doxorubicin. **Conclusions:** These results suggest that the acquisition of anoikis-resistance in human OS through AI growth alters proliferation and promotes chemoresistance, while resulting in unique and distinct expression profile changes. Targeting epigenetic regulation of this process may be a viable therapeutic strategy.

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Uropathogenic Escherichia coli Superinfection Enhances the Severity of Mouse Bladder Infection

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Urinary tract infections (UTIs) afflict over 9 million women in America every year, often necessitating long-term prophylactic antibiotics. One risk factor for UTI is frequent sexual intercourse, which dramatically increases the risk of UTI. The mechanism behind this increased risk is unknown; however, bacteriuria increases immediately after sexual intercourse, suggesting that physical manipulation introduces periurethral flora into the urinary tract. The majority of UTI are caused by Uropathogenic E. coli (UPEC), which are able to invade mouse and human bladder tissue and replicate in the cytoplasm to form clonal intracellular bacterial communities (IBCs). IBCs are necessary for bacteria to expand in number and replicate in the bladder lumen. Certain strains of mice develop persistent active infection called chronic cystitis that depends on intracellular bacterial replication and the host immune response. We hypothesized that frequent sexual intercourse increased the risk of UTI by introducing bacteria into an inflamed urinary tract allowing intracellular and extracellular niche access. We therefore investigated whether superinfection (repeat introduction of bacteria) resulted in increased risk of severe UTI, manifesting as persistent bacteriuria, high titer bladder bacterial burdens and chronic inflammation, an outcome referred to as chronic cystitis. Chronic cystitis represents unchecked luminal bacterial replication and is defined histologically by urothelial hyperplasia and submucosal lymphoid aggregates, a histological pattern similar to that seen in humans suffering chronic UTI. C57BL/6J mice are resistant to chronic cystitis after a single infection; however, they developed persistent bacteriuria and chronic cystitis when superinfected 24 hours apart. Elevated levels of interleukin-6 (IL-6), keratinocyte cytokine (KC/CXCL1), and granulocyte colony-stimulating factor (G-CSF) in the serum of C57BL/6J mice prior to the second infection predicted the development of chronic cystitis. These same cytokines have been found to precede chronic cystitis in singly infected C3H/HeN mice as well as correlated with increased rates of recurrent UTI when found in women suffering an acute UTI. Furthermore, inoculating C3H/HeN mice twice within a six-hour period doubled the proportion of mice that developed chronic cystitis. Intracellular bacterial replication, regulated hemolysin (HlyA) expression, and caspase 1/11 activation of the inflammasome were essential for this increase. Microarrays conducted at four weeks post inoculation in both mouse strains revealed upregulation of IL-1 and antimicrobial peptides during chronic cystitis. These data suggest a mechanism by which caspase-1/11 activation and IL-1 secretion could predispose certain women to recurrent UTI after frequent intercourse, a predisposition predictable by several serum biomarkers in two murine models.

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Identification of an Innate Lymphoid Cell Precursor in Human Secondary Lymphoid Tissue

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Innate lymphoid cells (ILCs) were only recently characterized as important immune effector cells that are also critical for the formation of secondary lymphoid tissues (SLT). Mature ILCs are divided into 3 distinct subpopulations, known as ILC1, ILC2, and ILC3, which produce gamma interferon, IL-13, and IL-22, respectively. However, how these cells develop is still unknown, and identification of a committed precursor in humans has yet to be discovered. Our laboratory first discovered a unique CD34+CD45RA+ multi-potent progenitor population in human SLT, specifically in tonsils. Herein we further dissect a novel subset of these CD34+ cells in SLT that co-express CD117 and the IL-1 receptor (IL-1R1). This CD34+CD117+IL-1R1+ population expresses each of the transcription factors associated with the three mature ILC subsets, including TBET, GATA3, RORα, RORγt, and AHR. In addition, using flow cytometry we have determined that this progenitor population expresses many cell surface molecules shared by ILCs, such as CD161 and CCR6. Together these data suggest a close relationship between this progenitor population and the mature ILCs. Indeed, in vitro culture experiments using purified populations of the CD34+CD117+IL-1R1+ subset demonstrate that these cells are capable of differentiating into each of the three ILC populations, further suggesting its role as an immediate precursor to the ILCs. Collectively, we have identified a novel subset of CD34+CD45RA+ human progenitor cells that selectively resides within SLT and is capable of generating ILC1, ILC2, and ILC3 cells in vitro, suggesting its role as a common ILC precursor.

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The Gut Microbiota Alters Chromatin and Enhancer States

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The gut microbiota has myriad impacts on physiology, metabolism, and immunity, and may play a pivotal role during early growth and development. Stressors such as early childhood nutritional insecurity—in combination with an immature gut microbial community configuration—may predispose to the development of childhood undernutrition. Although current therapeutic food interventions have resulted in dramatic reductions in mortality in children with moderate and severe undernutrition, they have not produced durable rescue of stunting, neurodevelopmental abnormalities, or immune defects. One possibility is that the gut microbiota induces prolonged, persistent host phenotypes through modifications of the epigenome. Therefore, we hypothesized that microbial colonization of the gut guides the establishment of epigenetic regulatory factors that persistently shape host phenotypes and responses to stressors. This may imply that early postnatal assembly and establishment of the microbiota is critical for host imprinting and development. To address these questions, we assayed the chromatin state of multiple discrete cell populations in germ-free mice, in germ-free mice that were colonized following the suckling-weaning transition with a microbial community, and in conventionally-raised control mice. Our study design allowed us to interrogate the effects of the microbiota during early development and after an initial developmental window of colonization. We chose to investigate tissue-specific chromatin changes using ATAC-seq, a method capable of analyzing small, discrete cell populations. We characterized purified, homogeneous populations that had direct exposure to the gut microbiota (Kupffer cells in the liver, TCR-αβ and TCR-γδ small intestinal intraepithelial lymphocytes), and circulating populations of peripheral blood CD4+ and CD8+ T-cells. Our analyses identified numerous tissue and lineage-specific enhancers, including both super enhancers and stretch enhancers. Additionally, we adapted a negative binomial-based approach to identify differentially enriched regions between and within experimental groups. We identified a number of reproducibly differentially expressed regions of the mouse genome that may reflect epigenetic modifications driven by the gut microbial community.

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Testosterone, Thrombophilia, Thrombosis

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We screened for previously undiagnosed thrombophilia (V Leiden-Prothrombin mutations, Factors VIII, XI, homocysteine, antiphospholipid antibody syndrome [APL]) in 15 men and 2 women with venous thromboembolism (VTE) or osteonecrosis (ON) 7 months (median) after starting testosterone therapy (TT) gel (30-50 mg/day) or intramuscular (100-400 mg/week) or HCG (6000 IU/week). Thrombophilia was studied in 2 healthy control groups without thrombosis (97 normals, 31 subjects on TT,) and in a 3rd control group (n=22) with VTE, not on TT (NTT-VTE). Of the 17 cases, 76% had ≥1 thrombophilia vs 19% of 97 normal controls (p<.0001), vs 29% of 31 TT controls (p=.002). Cases differed from normal controls by Factor V Leiden (12% vs 0%, p=.021), by high Factor VIII (>150%) (24% vs 7%, p=.058), by high homocysteine (29% vs 5% p=.007), and from both normal and TT controls for APL (18% vs 2%, p=.023, vs 0%, p=.04). Despite adequate anticoagulation, with TT continued after the 1st DVT-PE, one man sustained 3 DVT-PEs 5, 8, and 11 months later; a 2nd man had 2 DVT-PEs, 1 and 2 months later. Of the 10 cases with serum T measured on TT, 6 (60%) had supranormal T (>800 ng/dl) and of 9 with estradiol measured on TT, 7 (78%) had supranormal levels (>42.6 pg/ml). TT interacts with thrombophilia leading to thrombosis. TT continuation in thrombophilic men is contraindicated because of recurrent thrombi despite anticoagulation. Screening for thrombophilia before starting TT should identify men at high risk for VTE with an adverse risk/benefit ratio for TT.

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Blocking Airway Surface Liquid Acidification Improves Bacterial Host Defense in Cystic Fibrosis

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Cystic fibrosis (CF) disrupts airway host defense leading to bacterial lung infections, the major cause of mortality. Newborn humans with CF and CFTR $^{-/-}$ piglets, exhibit an abnormally acidic airway surface liquid (ASL), the thin layer of fluid lining the airways. The abnormally acidic ASL inhibits the activity of antimicrobial peptides, predisposing to bacterial infections. ASL acidification occurs, in part, due to the loss of CFTR-mediated HCO_3^- secretion into the ASL. However, CF ASL pH is acidic relative to serum, suggesting that airway epithelia secrete H^+. Inhibiting H^+ secretion may be a means to reduce the abnormal acidification and rescue the bacterial host defense defect. The goal of this study was to 1) identify the protein(s) secreting H^+ into ASL and 2) test if increasing ASL pH by inhibiting H^+ secretion would improve bacterial killing. We studied newborn CF pigs to avoid secondary consequences of infection and inflammation. To identify the mechanism of H^+ secretion, we tested inhibitors of V-ATPase, gastric H^+/K^+ ATPase, non-gastric H^+/K^+ ATPase, voltage-gated H^+ channels, and Na^+/H^+ exchangers, that previous studies suggested might be involved in ASL acidification. Only apical ouabain, an inhibitor of the non-gastric H^+/K^+ ATPase, increased ASL pH. Using siRNA to knockdown the non-gastric H^+/K^+ ATPase also increased ASL pH in CF and non-CF epithelia. Using pH-stat, we found that CF and non-CF porcine epithelia secreted H^+ at similar rates, and apical ouabain inhibited H^+ secretion in both genotypes. cAMP, which activates the non-gastric H^+/K^+ ATPase, further acidified ASL in CF and in non-CF. Apical ouabain and siRNA also blocked cAMP-stimulated H^+ secretion. To test the hypothesis that inhibiting the non-gastric H^+/K^+ ATPase would increase ASL pH and improve bacterial killing, we applied ouabain onto the tracheal surface of newborn pigs *in vivo*. Ouabain increased ASL pH and killing of *Staphylococcus aureus,* a common CF pathogen. Aerosolizing ouabain onto the apical surface of CF and non-CF porcine airway epithelial cell cultures increased ASL pH and enhanced bacterial killing, as well. We also tested the effect in humans by aerosolizing ouabain onto the nasal epithelia and found that it increased ASL pH *in vivo*. These findings identify the non-gastric H^+/K^+ ATPase as the primary pathway for acidifying porcine ASL. Furthermore, blocking H^+ secretion increased ASL pH and enhanced bacterial killing in CF pigs *in vitro* and *in vivo* and in humans *in vivo*. Consequently, therapies targeting the non-gastric H^+/K^+ ATPase might improve bacterial killing in CF and other diseases where ASL is abnormally acidic.

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Light Attenuating Artificial Iris - Implantable and Contact Lens Designs

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Purpose: Excessive light exposure leads to photophobia, glare and poor vision in patients with congenital or trauma induced iris damage. Commercial artificial irises provide aesthetics without restoring the natural iris's dynamic response to light. Novel artificial irises were developed in both implantable and contact lens forms using a photochromic material within a biocompatible polymer matrix. Photochromic materials activate by light to change opacity and diminish light transmission. **Methods:** The implantable artificial iris was created by reshaping Photopia, a photo-responsive material in polyethylene (Matsui International), into annular disks and spin-coating a layer of polydimethylsiloxane (PDMS, Sylgard 184, Sigma) on both sides. Another photochromic material, DEA powder, dissolved in acetone was combined with PDMS and underwent a 4-day wash cycle to form the artificial iris contact lens. Optical properties including the kinetics of activation and reversal, and percent light attenuation were measured using an UV/Vis Spectrophotometer for wavelengths 300-700nm. Both prototypes were subjected to in vitro cell toxicity experiments for up to 1 month. Cells were stained with a live/dead viability assay and imaged using confocal microscopy. Potential leaching of photo-responsive materials from the prototypes was quantified. **Results:** The implantable artificial iris showed activation by UV and blue light in 5 seconds and reversal in 1 minute with graded attenuation up to 40% of visible and 60% of UV light. 30% cell death was seen with exposure to Photopia alone but no significant death compared to control for the implantable artificial iris. NMR determined no apparent leakage of potentially toxic Photopia from the device. The artificial iris contact lens demonstrated activation and reversal of DEA by blue light in 1 second with absorption of 40% of incident light in both the UV and visible light range. 90% cell viability with normal morphology and proliferation was observed for cells exposed to the contact lens. Degradation studies showed leaching of less than 0.5% DEA. **Conclusions:** The combination of a photochromic material in a PDMS polymer matrix provides a new artificial iris design. Our implantable and contact lens artificial irises mimic natural iris functionality with their quick reversible activation to attenuate light entering the eye. The implantable artificial iris provides permanent relief from the adverse effects of iris damage such as light sensitivity. Whereas, the contact lens version allows patients selectivity in usage and aesthetics while maintaining dynamic light attenuation. In vitro cell experimentation and leaching studies established biocompatibility. Our photochromic artificial irises may provide an improved treatment option for patients with reduced iris functionality.

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Clostridium Difficile Pancolitis Caused by Antineoplastic Therapy

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Introduction: Clostridium difficile is an etiologic agent for antibiotic-associated diarrhea (15%–25% of all cases) and pseudomembranous colitis (95%–100% of all cases). Literature shows that Clostridium difficile colitis is not a common cause of diarrhea in cancer patients treated with antineoplastic chemotherapy. It however needs to be considered in any cancer patient on chemotherapy who presents with diarrhea. We report a case of 80 yr old female on chemotherapy who developed watery diarrhea and stool was positive for Clostridium difficile toxin. **Case Report:** An 80 yr old caucasian american lady with past medical history of hypertension, diabetes mellitus, coronary artery disease, metastatic pancreatic cancer was admitted to our hospital with painless watery stools for five days. She was on a chemotherapy regimen with oxaliplatin/fluorouracil/irinotecan. Her last dose of chemotherapy was one week prior to presentation. At the time of admission she was afebrile, vitally stable, in mild distress due to frequent bowel movements. Abdominal Examination revealed increased bowel sounds with mild tenderness on deep palpation and no peritoneal signs. She denied any melena or hematochezia. Labs revealed severe neutropenia with stools positive for Clostridium difficile toxin. CT scan of the abdomen was done which revealed circumferential wall thickening and inflammation involving entire colon. She denied any use of antibiotics in the past 2 months. She was managed with IV fluids, antiemetics and started on oral vancomycin. She also received neupogen and her blood counts stabilized. Her admission was complicated by ileus of the small intestine requiring addition of IV metronidazole. She had a protracted hospital stay of 15 days. **Discussion:** Colitis without diarrhea and diarrhea due to chemotherapy is commonly recorded in literature. Clostridium associated diarrhea [CDAD] has been linked more commonly with hematologic antineoplastics such as cytarabine. Solid tumor antineoplastics are less likely to cause prolonged neutropenia, thereby reducing risk of patients developing CDAD or colitis. Despite this fact, it should always be considered as a cause for diarrhea in this patient group. Prompt diagnosis and aggressive supportive care with IV hydratation, broad-spectrum antibiotics, and close surgical monitoring for selective intervention can significantly decrease the morbidity and life-threatening complications associated with this infection.

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Atypical Chromatin Binding by the Histone Methyltransferase ASH1L

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ASH1L is a histone methyltransferase originally discovered in D. melanogaster as a member of the Trithorax group of proteins, loss of which results in misregulation of Hox and Wnt gene expression. Studies also show its general role in the regulation of transcriptionally active genes. ASH1L contains three C-terminal putative histone binding domains: a bromodomain, PHD finger, and BAH domain. Strikingly, the bromodomain lacks a conserved Asn residue in the binding pocket and cannot bind acetylated lysine, the property for which almost all other acetyl-lysine binding bromodomains have been characterized. We've classified this bromodomain as non-canonical and are searching to identify its biological target. Our work has identified the PHD finger as a reader of methylated H3K4, a mark that is typically associated with gene activation and is likely responsible for binding of this protein to regions rich in this histone mark. We report the structure of the Bromo-PHD tandem module in complex with H3K4me2 by NMR. The structure reveals the molecular determinants of H3K4me2 recognition. It also reveals that the two domains operate independently of each other. We are using a number of structural, genetic, and biochemical techniques to define the role of ASH1L in Hox and global gene expression, including complex identification by mass spectrometry and CRISPR gene editing. This process is expected to yield the identity of a biological ligand for the ASH1L bromodomain. Numerous diseases are caused by misregulation of Hox gene expression, including leukemias, infertility, and defects in body patterning. Identification of the roles that the ASH1L tandem domains play in transcription, therefore, may reveal new mechanisms and insight into the way this misregulation occurs.

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Massively Parallel Cis-Regulatory Analysis in the Mammalian Central Nervous System

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Although coding mutations have been the focus of medical genetics, the vast majority of disease-associated variants are non-coding. Variants that fall within *cis*-regulatory elements (CREs, i.e., promoters, enhancers, and silencers) can alter gene expression and thereby contribute to disease. However, high-throughput functional approaches for deciphering CREs in disease-relevant tissues *in vivo* are lacking. Recently, massively parallel reporter assays (e.g., CRE-seq) have been developed, in which barcoded plasmid reporters are introduced into cells and the resulting expression levels are quantified by next-generation sequencing. Here, we utilize CRE-seq with two major advances. First, we extend the size of CREs that can be readily assayed by using a capture-based approach, in which we

adapt exome capture technology to instead capture putative CREs from individual genomes. The captured fragments tile across target regions, allowing for genome-wide truncation mutation analysis. Second, we use adeno-associated virus (AAV) to deliver the CRE-seq libraries into two mouse tissues of the central nervous system, the cerebral cortex and the retina. We not only demonstrate tissue-specific CRE activity but also functionally parse CREs to identify critical regions. Our approach may be extended to a wide range of CREs, tissues, and model systems, including non-human primates and human induced pluripotent stem cell (iPSC)-derived organoid cultures. As whole-genome sequencing becomes routine, our approach promises to greatly accelerate the interpretation of the thousands of non-coding variants identified in patients.

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Inhibiting VEGF Signaling by Targeting Receptor Endocytosis

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Vascular Endothelial Growth Factor (VEGF) is important for endothelial cell proliferation and migration, and signals through cognate cell surface receptor tyrosine kinases (VEGFRs). VEGF-induced VEGFR internalization is known to be essential for signal amplification, yet the mechanisms coordinating the trafficking and intracellular signaling of VEGFR remain unknown. We show that VEGFR constitutively binds the guanine nucleotide exchange factor, GEP100, and in the presence of VEGF, the VEGFR-GEP100 complex activates ADP-ribosylation factor 6 (ARF6). ARF6 activation promotes binding of VEGFR to its co-receptor neuropilin, internalization of the receptor, and amplification of receptor tyrosine kinase (RTK) signaling cascades. We identify a small molecule inhibitor of ARF6 that blunts internalization of VEGFR, prevents its immediate downstream signaling, and mutes pathologic endothelial hyperpermeability in mouse models of diabetic retinopathy. This work demonstrates a VEGFR-neuropilin-GEP100-ARF6 cascade controls VEGFR internalization and signaling, and suggests a therapeutic strategy for inhibiting RTK activity by interfering with receptor endocytosis.

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Development of Poly(ethylene glycol) Hydrogels for Salivary Gland Regeneration

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Over 40,000 patients annually in the U.S. are diagnosed with head and neck cancers. Most of these patients undergo radiation treatment resulting in permanent dry mouth, a condition known as xerostomia. Xerostomia adversely affects oral hygiene, diet, and speech production. Direct injection of primary submandibular gland cells (SMG) prepared as multicellular aggregates known as "salispheres" by suspension culture into irradiated mouse salivary glands can promote gland regeneration. However, the mechanism of gland regeneration is not well understood and suspension culture provides a limited way to study salispheres *in vitro*. In order to further characterize salispheres, and to improve their *in vivo* regenerative potential, we are investigating the use of poly(ethylene glycol) (PEG) as a 3D culture system for salispheres. PEG hydrogels are synthetic and photopolymerizable tissue engineering scaffolds with tunable stiffness, degradability, and bioactivity. Salispheres were encapsulated within PEG hydrogels using thiol-ene polymerization. Upon encapsulation salispheres showed viability comparable to suspension culture (70-80%), and proliferation over a 14 day culture period resulting in microtissue structures up to 200 μm in diameter. Encapsulated salispheres degraded PEG hydrogels designed with matrix metalloproteinase (MMP) degradable crosslinks, resulting in increased rates of cell liberation from the hydrogels. Additionally, encapsulation of salispheres within MMP-degradable hydrogels increased secretion of α-amylase, a marker of functional gland tissues, indicating that modification of hydrogel properties can influence the function of encapsulated salispheres. In conclusion, PEG hydrogels provide a highly-modifiable scaffold that supports salisphere viability, proliferation, and function and may prove to be advantageous for salivary gland tissue engineering.

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Lung Cancer Incidence Decreases with Elevation: Evidence for Oxygen as an Inhaled Carcinogen

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The atmospheric concentration of oxygen, a driver of free radical damage and tumorigenesis, decreases sharply with rising elevation. To understand whether ambient oxygen concentrations play a role in human carcinogenesis, we characterized age- adjusted cancer incidence (compiled by the National Cancer Institute from 2005–2009) across counties of the elevation-varying Western United States and compared trends displayed by respiratory cancer (lung) and non-respiratory cancers (breast, colorectal, and prostate). To adjust for important demographic and cancer-risk factors, 8–12 covariates were considered for each cancer. We produced sensible regression models that captured known risks. Models demonstrated that elevation strongly, negatively associates with lung cancer incidence ($p < 10^{-16}$), but not with incidence of non-respiratory cancers. For every 1000 meter rise in elevation, lung cancer incidence decreased by 7.23 [99% CI: 5.18–9.29] cases per 100,000 individuals, equivalent to 12.7% of the mean incidence, 56.8. As a predictor of lung cancer incidence, elevation was second only to smoking prevalence in terms of significance and effect size. Furthermore, no evidence of uncontrolled confounding or ecological fallacy was detected: the lung cancer association was robust to varying regression models, county stratification, and population subgrouping; additionally seven environmental correlates of elevation, such as exposure to sunlight and fine particulate matter, could not capture the association. Overall, our findings suggest the presence of an inhaled carcinogen inherently and inversely tied to elevation, offering epidemiological support for oxygen-driven tumorigenesis. Finally, highlighting the need to consider elevation in studies of lung cancer, we demonstrated that previously reported inverse lung cancer associations with radon and UVB became insignificant after accounting for elevation.

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Trypanosome RNA Editing Alignment Tool: Using Deep Sequencing to Understand the Roles of Accessory Factors in Kinetoplastid RNA Editing

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In *Trypanosoma brucei*, RNA editing is an essential process whereby mitochondrial mRNAs are altered to their final coding form through the specific addition and deletion of uridines using guide RNAs (gRNAs) as templates. Editing of most mRNAs requires the sequential utilization of multiple gRNAs. Though the enzymatic components of this process have been identified, there are many accessory factors that are essential for RNA editing but whose roles are not well understood. These include many components of the Mitochondrial RNA Binding Complex 1 (MRB1). By analyzing the characteristics of partially edited mRNA sequences in the presence and absence of specific proteins, we can examine the effect of these proteins on editing at the sequence level for the first time. Changes in the partially edited sequences upon depletion of accessory editing factors will elucidate the roles these proteins play in the orchestration of editing. To analyze the partially edited sequences from any given cell line, we have created the Trypanosome RNA Editing Alignment Tool (TREAT). This multiple alignment program allows us to examine large deep sequencing data sets of partially edited mRNAs and determine the regions which are fully edited, pre-edited and mis-edited for each individual sequence and study trends across whole populations of partially edited mRNAs. Cross-referencing these data with existing gRNA datasets provides insight into the utilization of specific gRNAs. Our laboratory previously showed that depletion of the MRB1 component, TbRGG2 impacts the 3′ to 5′ progression of editing. Thus, our initial studies are focused on three components of the TbRGG2 subcomplexes, namely TbRGG2, MRB8170/4160, and MRB8180. Analysis of *T. brucei* cell lines depleted of each of these factors using TREAT will not only lead to a deeper understanding of the role of these accessory editing factors but will reveal a great deal about the nature of the RNA editing process in trypanosomes.

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Differential Neural Responses to Palatable Food in Binge Eating Prone and Binge Eating Resistant Female Rats

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Introduction: Binge eating is the core, maladaptive behavior observed in the most prevalent types of eating disorders that affect women. Binge eating is defined as an excessive consumption of highly palatable (sweet and fat) foods (PF). The mesocorticolimbic reward circuit, including the nucleus accumbens (NA) and the prefrontal cortex (mPFC), responds to the pleasurable, hedonic properties of PF, thus binge eating is considered to be a reward-driven behavior caused by pathological over-activation of this circuit during PF intake. Using a well-established animal model of binge eating proneness, the objective of this study was to determine if the neural response to PF in the mesocorticolimbic circuit differs as a function of binge eating phenotype. **Methods:** Female Sprague-Dawley rats (n=40) underwent three weeks of feeding tests consisting of *ad libitum* access to chow and water and an additional 4 hours of access to PF presented three days per week. Total 4-hour PF intake was divided into top, middle, and bottom tertiles on each day when the PF was presented. Binge eating prone (BEP) rats were those consistently scoring in the top tertile (i.e., 50%) and never in the bottom tertile of PF intake, while binge eating resistant (BER) rats were those consistently scoring in the bottom tertile and never the top tertile of PF intake. One week after the final feeding test, BEP and BER rats were exposed to PF for one hour prior to sacrifice to induce Fos expression, a marker of neural activity. Fos expression was stereologically analyzed in the NA core and NA shell as well as the cingulate, prelimbic, and infralimbic cortices of the mPFC. Analysis of covariance (ANCOVA) models, using total amount of PF consumed at sacrifice as the covariate, were used to determine if total Fos expression in the NA and mPFC differed between BEP and BER rats. **Results:** BEP rats showed a higher Fos expression than BER rats in all regions of the NA and mPFC. ANCOVA models demonstrated statistical trends in the NA core [$F(1,8)=3.00$, $p<0.1$] and NA shell [$F(1,8)=1.91$, $p<0.1$], as well as statistically significant differences in the cingulate cortex [$F(1,8)=7.75$, $p<0.01$], the prelimbic cortex [$F(1,8)=19.26$, $p<0.01$] and the infralimbic cortex [$F(1,8)=6.94$, $p<0.05$]. **Conclusion:** These data suggest that a significantly enhanced mesocorticolimbic response to PF is an important neural correlate of binge eating proneness. Furthermore, these data suggest that individual differences in the neural response to PF might contribute significantly to binge eating behaviors in women.

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Overcoming Temozolomide Resistance in Glioblastoma Using MGMT-Targeting Spherical Nucleic Acids

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Glioblastoma multiforme (GBM) is the most prevalent primary central nervous system malignancy. Due to the aggressive nature of these tumors and our inability to adequately treat them, only 3-5% of patients survive longer than 3 years post-diagnosis. The standard of care for newly diagnosed GBM is surgical resection followed by adjuvant radiotherapy and temozolomide (TMZ) chemotherapy. TMZ cytotoxicity is mediated primarily through methylation of the O^6-position of guanine. In the majority of patients, this methyl group is rapidly removed by the enzyme O^6-methylguanine-DNA methyltransferase (MGMT), conferring resistance to the chemotherapy. However, in a small subset of GBM patients the promoter region for MGMT is methylated over the course of tumor development. This epigenetic silencing of MGMT activity allows TMZ to induce apoptosis in glioblastoma cells and drastically increases survival in GBM patients. The present study seeks to recapitulate this improved survival phenotype by combining TMZ with a novel nanoconstruct capable of silencing MGMT expression. The nanoconstruct consists of gold nanoparticles densely conjugated with small interfering RNA (siRNA) duplexes designed against MGMT (MGMTi-Spherical Nucleic Acids (SNAs)) and has been found to have unique characteristics, including (1) the rapid internalization by all glioma cell types studied including tumor neurospheres, (2) the capacity to potently silence MGMT expression, (3) increased apoptotic response in GBM cells, (4) the ability to cross the blood-brain barrier (BBB), blood-tumor barrier (BTB), and accumulate in GBM xenografts, and (5) no observable acute toxicity at high doses in animal models. In summary, preliminary data suggest MGMTi-SNAs sensitize GBM cells *in vitro* and *in vivo*, enhancing the therapeutic response to TMZ.

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Adapting Insulin Signaling

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Adaptor proteins play an essential role in regulation of signaling and metabolism. Deletion of the adaptor protein p66Shc improves glucose tolerance in obese mice and confers resistance against diabetes. Using mass spectrometry analysis, we observed that deleting p66Shc resulted in increased glycolytic metabolism. This observation was confirmed in fibroblasts lacking p66Shc with restored p66Shc expression, suggesting that p66Shc might inhibit glucose metabolism under physiological conditions. Biochemical assays analyses suggested that p66Shc acts as a negative regulator of the nutrient-sensing Insulin-PI3K-mTOR signalling pathway, which could potentially explain the inhibitory effect of p66Shc on glycolysis. In summary, our data indicate that loss of p66Shc redirects glucose carbon towards anabolic metabolism, and this effect is mediated, in part, through the insulin-PI3K-mTOR pathway. Manipulating the abundance of p66Shc or interfering with p66Shc function could be used to treat diseases characterized by disruptions in PI3K-mTOR pathway, including diabetes and cancer.

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Colonic Foxp3+RORγt+ T Cells Recognize a Unique Set of Mucosal Antigens

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CD4+ T cells play a central role maintaining tolerance towards commensal organisms in mucosal tissues. While both regulatory T(reg) and T helper (Th)17 cells are enriched in the mucosal tissue of healthy individuals, it has been suggested that unbalanced conversion of T cells from a Treg cell phenotype toward a Th17 phenotype can lead to colitis. The phenotypes of Treg and Th17 cells are determined by the expression of the lineage defining transcription factors Foxp3 and RORγt, respectively. Despite the ability of these two transcription factors to induce opposing phenotypes and directly inhibit one another, a substantial population of mucosal CD4+ T cells co-expresses Foxp3 and RORγt. In order to address how these cells come to express two opposing transcription factors, we have utilized T cell receptor (TCR) sequence analysis to investigate their development. Our data indicates that Foxp3+ RORγt+ cells possess a unique TCR repertoire, suggesting that they develop in response to a distinct set of antigens. The absence of many of these TCR specificities in germ-free (GF) mice suggests that they recognize antigens derived from commensal bacteria. Using an *in vivo* screen, we have identified a TCR clone that reliably mediates acquisition of the Foxp3+ RORγt+ phenotype. Using a TCR transgenic mouse expressing this TCR, we have begun to evaluate the developmental origin and function of mucosal Foxp3+RORγt+ cells, particularly in relation to conventional Treg and Th17 cells.

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Effects of Naturally Occurring Mutant HCV NS3:1406-1415 Mutant Epitopes on pMHC/TCR Binding and Functional Recognition by HCV TCR Gene-Modified T Cells

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Hepatitis C virus (HCV) infects approximately 3% of the world's population, often causing associated diseases including cirrhosis and hepatocellular carcinoma (HCC). Chronic infections leading to cancer are thought to be attributed to the rapidly mutating HCV genome leading to immune escape variants. A novel, immune-based approach for targeted therapy utilizes adoptive transfer of autologous, genetically modified T cells. Our lab has firmly demonstrated its ability to use recombinant retroviral vectors encoding T cell receptor (TCR) genes to redirect the specificity of normal peripheral blood lymphocyte (PBL)-derived T cells to recognize tumor and viral antigens. We have previously identified and cloned a novel, high affinity TCR from an HLA-A2-restricted, HCV NS3:1406-1415-reactive T cell clone. Here, we demonstrate PBL-derived T cells transduced with this TCR can recognize peptide-loaded targets and HCV^+ HCC cells in a CD8-independent manner. We have measured the affinity of the TCR/pMHC complex and have structural models of this interaction. Transduced T cells can also recognize tumors expressing an epitope-specific minigene or full length NS3 protein, indicating this TCR can recognize naturally processed antigen. Moreover, T cells engineered with this receptor can recognize a variety of naturally occurring mutant HCV antigens in the context of peptide-loaded targets, minigene-expressing tumors, and naturally processed antigen, demonstrating that this TCR has a unique crossreactivity. Both $CD4^+$ and $CD8^+$ T cells can be lytic and/or multi-cytokine-secreting in varying combinatorial proportions against wildtype antigen and various mutants. Furthermore, adoptive transfer can mediate regression of human HCC tumors in a mouse xenograft model. Based on these observations, we believe that despite the instability in the HCV genome, TCR gene modified T cells reactive against mutagenic HCV antigens may be a promising clinical therapy for treating HCV infection and associated HCC.

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Partial Trisomy 2p in a Patient with CHARGE Syndrome

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Tight regulation of gene expression is dependent on interactions of transcription and chromatin architecture. CHD7, an ATP-dependent chromatin remodeler, is required for proper development of tissues affected in CHARGE syndrome, a multiple anomaly condition associated with craniofacial dysmorphisms and inner ear dysfunction. While mutations in *CHD7* occur in the majority of individuals with CHARGE, other genetic lesions in the remaining patients have not been well defined. We identified a child with CHARGE syndrome and trisomy 2p, a phenotypically heterogeneous disorder variably affecting the central nervous system, craniofacial structures, special sensory systems, and heart. Our patient had a complex chromosomal gain included a partial duplication and partial triplication that spanned 6.5 Mb including 24 genes, one of which is the HMG box transcription factor *SOX11*. While over 100 cases of partial trisomy 2p have been reported, our case represents only the fourth reported case presenting with *de novo* tandem duplication of 2p and no other relevant genetic abnormalities. Recent molecular evidence suggests that CHD7 is necessary for proper expression of SOX11 during neurogenesis. Mice haploinsufficient for *Sox11* exhibit developmental defects in tissues commonly affected in partial trisomy 2p and CHARGE syndromes. Based on these observations, we hypothesized that CHD7 and SOX11 may cooperate to promote proper development of CHARGE-related tissues and sought to determine whether altered dosage of *SOX11* contributes to phenotypes observed in CHARGE syndrome. We performed qPCR and Sanger sequencing to test whether other *CHD7*-mutation negative CHARGE individuals also have altered dosage of or mutations in *SOX11*. No *SOX11* duplications or coding mutations were found in 28 unrelated, CHD7 mutation-negative CHARGE individuals. Changes in *Sox11* expression in microdissected otocysts from E10.5 wildtype and *Chd7* mutant mice were assayed using RNA-seq, and confirmed by qRT-PCR. *Sox11* mRNA was significantly reduced in *Chd7* null otocysts relative to wild type and heterozygous mice. Immunofluorescence with antibodies against CHD7 and SOX11 on embryonic mouse tissues demonstrated co-localization in neural, nasal, inner ear, and ocular tissues. Taken together, these observations suggest that dosage of *SOX11* is critical for development of CHARGE-relevant tissues in both mice and humans, and that CHD7 may govern proper *Sox11* expression across development. Furthermore, this emerging pathway may account for a significant proportion of the pleiotropic effects of CHD7 during development. Further functional studies using mice and zebrafish are underway to help clarify the molecular and genetic relationships between SOX11 and CHD7, and to further dissect the relative importance of SOX11 in CHARGE.

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Novel Interaction Between EGFR and PARP1 may Modulate DNA Repair in Human Triple Negative Breast Cancer

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Background/Objectives: Few therapeutic options are effective for triple negative breast cancers (TNBCs), thus necessitating novel approaches. We recently reported a novel synthetic lethality with combined EGFR and PARP inhibition, due to a homologous recombination (HR) repair defect induced by EGFR inhibition. The mechanism by which EGFR regulates HR DNA repair, however, is not well characterized. As EGFR has no DNA binding domain, we hypothesize it may exert its effects on repair through nuclear translocation and interaction with essential repair proteins, such as PARP1, and thereby sensitize TNBC cells to the combination of EGFR and PARP inhibition. **Methods:** The TNBC cell lines MDA-MB-231 and MDA-MB-468 were used as TNBC models *in vitro*. EGFR inhibition was achieved through the treatment of cells with 1uM lapatinib. MALDI-TOF mass spectroscopy was utilized to ascertain protein-protein interactions. Confirmation of interaction data and determination of subcellular location was accomplished through immunoprecipitation and subcellular fractionation, Duo-link *in situ* proximity ligation, and *in vitro* binding assays. To determine if interactions were dependent on DNA, Ethidium Bromide and DNAse were used. Kinetics of EGFR subcellular localization was investigated via immunofluorescence. **Results:** Interestingly, mass spectroscopy identified a novel interaction between EGFR and PARP1. These interactions were not found upon EGFR inhibition with lapatinib. Furthermore, we validated the EGFR and PARP1 interaction with reciprocal immunoprecipitation, Duo-link, and *in vitro* protein binding assays. Additionally, these proteins were found to interact in the nucleus, independent of DNA. Lastly, DNA damage increased nuclear levels of EGFR, which was attenuated by lapatinib. **Conclusions:** EGFR may regulate DNA repair via interaction with the DNA repair protein, PARP1. Further investigation of how EGFR regulates DNA repair may shed light on novel roles of EGFR in the nucleus and uncover future targets that can be exploited in the treatment of TNBCs.

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CD8 T Regulatory Cells Attenuate Alloantibody Production and are Essential for Tolerance Induction to Islet Allografts

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Background: CD8 T Regulatory Cells (CD8 Tregs) have emerged as potent suppressors of islet allograft rejection. How CD8 Tregs mechanistically suppress alloimmunity remains unknown and they have yet to be harnessed therapeutically. We tested the hypotheses that CD8 Tregs suppress alloimmunity by dampening the Germinal Center response and that they are essential for allogeneic tolerance induced by anti-CD45RB therapy. **Methods:** Candidate cellular pathways for CD8 Treg (CD8+CD44+CD122+) action were identified by BD Lyoplate. Suppression of the antibody response was tested 1) following hapten-carrier immunization in RAG mice reconstituted with B cells, CD4 T cells, and CD8 Tregs or non-Tregs and 2) following C3H splenocyte challenge in CD8 Treg sufficient B6 vs. CD8 Treg deficient B6.IL-15$^{-/-}$ mice (IL-15 is required for survival of CD122+ CD8 Tregs). CD8 Treg activation following anti-CD45RB therapy was assessed by BrdU staining. To determine whether CD8 Tregs are required for transplant tolerance induction, diabetic B6 and B6.IL-15$^{-/-}$ mice were transplanted with allogeneic C3H islets, treated with anti-CD45RB, and monitored for rejection. **Results:** CD8 Tregs expressed increased Germinal Center markers CD24, CD38, PDL-1, and ICOS-L in addition to CD45RB suggesting that they actively regulate T and B cell collaboration and that they could be activated by anti-CD45RB immunotherapy. NP-KLH experienced CD8 Tregs dampened the high-affinity anti-NP IgG response and eliminated target CD4 T Follicular Helper cells. CD8 Treg deficient IL-15$^{-/-}$ mice generated greater anti-C3H IgG alloantibody responses vs. CD8 Treg sufficient B6 mice (>2.5 fold, $p<0.05$). Anti-CD45RB therapy doubled CD8 Treg proliferation and resulted in their 2-fold expansion ($p<0.005$). Whereas anti-CD45RB treatment induced long-term tolerance to C3H islet allografts in CD8 Treg sufficient B6 mice (MST - 13d untreated vs. >100d anti-CD45RB), anti-CD45RB therapy failed to induce tolerance to C3H islet allografts in CD8 Treg deficient IL-15$^{-/-}$ mice (MST - 21d untreated vs. 36 d anti-CD45RB). **Conclusions:** Harnessing CD8 Tregs as a cellular therapy for transplantation may prevent rejection by targeting previously activated lymphocytes that drive the anti-graft response. We demonstrate that these cells regulate the germinal center response, which limits alloantibody production and may impact acute and chronic rejection. We also demonstrate that these cells can be activated therapeutically by anti-CD45RB, which is the first therapy to target this novel cellular pathway. Overall, these findings form the foundation for incorporating CD8 Tregs into the graft-protective armamentarium.

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Persistent Gut Microbiota Immaturity in Malnourished Bangladeshi Children

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Childhood malnutrition, specifically under-nutrition, is a major health problem in Bangladesh and many other low-income countries. Therapeutic food interventions have reduced mortality in severe acute malnutrition (SAM) but incomplete restoration of healthy growth remains a major problem. The relationships between the type of nutritional intervention, the gut microbiota, and therapeutic responses are unclear. In the current study, bacterial species whose proportional representation define a healthy gut microbiota as it assembles during the first two postnatal years were identified by applying a machine-learning-based approach to 16S rRNA datasets generated from monthly fecal samples obtained from a birth-cohort of children, living in an urban slum of Dhaka, Bangladesh, who exhibited consistently healthy growth. These age-discriminatory bacterial species were incorporated into a model that computes a 'relative microbiota maturity index' and 'microbiota-for-age Z-score' that compare development ('maturation') of a child's fecal microbiota relative to healthy children of similar chronologic age. The model was applied to twins and triplets (to test for associations of these indices with genetic and environmental factors including diarrhea), children with SAM enrolled in a randomized trial of two food interventions, and children with moderate acute malnutrition (MAM). Our results indicate that SAM is associated with significant relative microbiota immaturity that is only partially ameliorated following the nutritional interventions. Immaturity is also evident in MAM and correlates with anthropometric measurements. Further integration of 16S rRNA datasets generated from monthly sampled infants and children from different study sites around the world, indicate that these observations of a developmental cascade of microbiota assembly are robust to geography and immaturity in malnourished children can be diagnosed using a model generated using healthy controls outside of Bangladesh. Microbiota maturity indices provide a microbial measure of human postnatal development, a way of classifying malnourished states, and a parameter for judging therapeutic efficacy. More prolonged interventions with existing or new therapeutic foods and/or addition of gut microbes may be needed to achieve enduring repair of gut microbiota immaturity in childhood malnutrition and improve clinical outcomes.

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Coordination of an Associative Memory Across Regions of the Medial Temporal Lobe

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Retrieval of new, but not old, associative memories requires multiple subregions of the medial temporal lobe system. We used trace eyeblink conditioning, a model of human declarative memory, to study the role of hippocampal CA1 and dentate gyrus (DG), as well as perirhinal (PR) and lateral entorhinal (EC) cortices, in acquisition and retention of associative memories. Information about conditioned and unconditioned stimuli reaches the hippocampus via projections from PR to EC. Within hippocampus, EC projects to both DG and CA1. Current cognitive models suggest that DG supports specificity of memory via pattern separation, while CA1 enables context-rich memory via pattern completion. The role of lateral entorhinal cortex and perirhinal cortex in trace conditioning are less well understood. By comparing neural responses between conditioned and pseudo-conditioned animals (that receive CS and US in an unpaired manner), we found that acquisition of an associative neural patterns differs between nodes of the MTL. Even before animals learned to consistently perform a conditioned eyeblink, neural responses evolve across each area as stimuli converge on the hippocampus. The results provide new insights into the gradual formation of a medial temporal lobe network supporting a task-specific associative memory.

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T Cell-Mediated Systemic Recruitment of Neutrophils and Monocytes After Intranasal Staphylococcal Enterotoxin A Exposure

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Staphylococcus aureus (S. aureus), a common pathogen colonizing the human nose and skin, is one of the most important causes of life-threatening infections. The high morbidity and mortality of *S. aureus* infections are ascribed to various virulence factors, including superantigens. Superantigens, such as staphylococcal enterotoxin A (SEA), mediate food poisoning and toxic shock syndrome and are also implicated in a number of pulmonary diseases and even sepsis. Their potency is embedded in their ability to directly bind to MHC II on antigen presenting cells and then crosslink with specific TCR Vβ chains on T cells (e.g. Vβ for SEA). The subsequent oligoclonal expansion and activation of T cells unleashes a massive immune response. Although SEA has been studied for decades, the exact mechanism of its action is not completely understood. We previously showed that intranasal administration of SEA in mice leads to a T-cell mediated recruitment of neutrophils and monocytes to the lungs. We hypothesized that the local migration of these innate cells to the lungs would be coincident with a systemic immune response. The aim of this study was to understand the early systemic responses to intranasal SEA exposure, and in particular, to illuminate the mechanism of how SEA-activated T cells orchestrate migration and activation of neutrophils and monocytes. Intranasal instillation of SEA in mice caused a rapid influx of neutrophils and monocytes to peripheral blood, followed by their recruitment to spleen and draining and non-draining lymph nodes. This migration was dependent on T

cells since TCR $\beta\delta^{-/-}$ mice were unable to recruit these cells unless they received an adoptive T cell transfer. Confocal imaging showed that the recruited neutrophils localized to the T cell zone within the lymph nodes. Utilizing qPCR and multiplex assays, an increased expression of several known neutrophil and monocyte chemotactic factors, including CXCL1, CXCL2, CCL2, CCL3, CCL4 and CCL7 was detected in the lymph node tissue as early as 1 h after SEA exposure. By sorting specific cell populations, we found that these chemokines were differentially expressed. Interestingly, CXCL1 and CXCL2 were mostly produced by dendritic cells while CCL3 and CCL4 were made by SEA-activated $V\beta3^{+}$ T cells. Our future studies will focus on illuminating the mechanism of chemokine release by T cells and dendritic cells. Understanding how SEA-activated T cells affect the migration and activation of these innate cells is not only important for designing countermeasures against SEA-impacted diseases but it will also enhance our knowledge of inflammatory mechanisms.

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Ethnic Differences in Cardiovascular Disease Mortality and Known Risk Factors in the Multiethnic Cohort

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Previous studies have demonstrated differences in cardiovascular disease (CVD) risk across racial/ethnic populations which cannot be fully explained by the prevalence of known risk factors. In this study we examined differences in risk of acute myocardial infarction (AMI), other heart diseases (OHD), and stroke by race/ethnicity, and whether or not known risk factors could explain these differences within the Multiethnic Cohort (MEC). This prospective analysis included ~139,000 African American, Latino, Japanese, Native Hawaiian and White individuals with ~5100 male CVD-related deaths and ~3500 female CVD-related deaths ascertained from 1993 to 2010. Primary risk factors examined included BMI, diabetes status, hypertension, smoking, education, intake of alcohol, cholesterol, saturated fat intake and other dietary factors (unsaturated fat, omega 3 ad omega 6 fatty acids, fiber from fruits/grains/legumes, sodium, folate, and isoflavones), as well as menopausal and HRT status in women. While models adjusted for known risk factors reduced mortality risk estimates for all endpoints versus Whites, large differences in AMI-associated mortality remained for Japanese American men (RR 0.66, 95% CI 0.53 – 0.82). For OHD-related death, compared to Whites, large disparities remained amongst African American men (RR 1.24, 95% CI 1.1-1.4), Native Hawaiian men (RR 1.57, 95% CI 1.34 – 1.84), and Japanese American men (men: RR 0.80, 95% CI 0.70-0.91) and women (RR 0.8, 95%CI 0.65-0.98). For stroke-related mortality, African American men and women had the highest risk (men: RR 1.59, 95% CI 1.29-1.96; women: RR 1.26, 95% CI 1.03-1.54). The risk factors with the greatest contribution to risk disparities by ethnicity were hypertension, education, diabetes and HRT use in women. In men, primary risk factors can account for most of the differences in CVD risk by ethnicity. Amongst women of Native Hawaiian and Japanese American ethnicity, the addition of nutritional factors to the model greatly reduces the differences in CVD risk by ethnicity. Additional unmeasured environmental factors, genetic risk factors, and other socioeconomic factors may account for the remaining disparities in risk.

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HSC-Independent Yolk Sac Progenitors Bear Hallmarks of JMML in a PTPN11D61Y Mouse Model

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Juvenile Myelomonocytic Leukemia (JMML) is a fatal pediatric myeloproliferative neoplasm (MPN) that develops *in utero*. JMML is caused by somatic mutations in Ras-Erk signaling genes, most commonly gain of function mutations in PTPN11, which cause growth hypersensitivity to the hematopoietic cytokine GM-CSF. Chemotherapy is not an effective JMML treatment and 50% of patients relapse following the only curative therapy – allogeneic hematopoietic stem cell (HSC) transplantation. The reasons for this markedly high relapse rate are unknown. Tissue macrophages are unique among hematopoietic cells in persisting after HSC transplantation. As such, their involvement in the pathogenesis of JMML could explain the observed ineffectiveness of HSC transplantation. Recent studies have shown tissue macrophages to descend from embryonic HSC-independent progenitors in the yolk sac. In this study, we sought to determine whether these HSC-independent yolk sac progenitors bear hallmarks of MPN in a mouse model of JMML. Using the Vav1 promoter-directed Cre recombinase, we generated a mouse model of JMML that expresses the $PTPN11^{D61Y}$ gain of function mutation in all waves of embryonic and adult hematopoiesis. $PTPN11^{D61Y/+}$; VavCre+ mice are viable, born at expected Mendelian ratios, and develop peripheral blood monocytosis as early as 4 weeks of age. E14.5 fetal liver progenitors from $PTPN11^{D61Y/+}$; VavCre+ embryos displayed GM-CSF hypersensitivity in colony forming assays at all measured doses of GM-CSF ($p<0.05$, n=7; all statistical analyses performed using two-tailed Student's t-test) and possessed hyperactive Ras-Erk pathway signaling by western blotting. Since the E14.5 fetal liver contains all waves of embryonic hematopoiesis, we restricted our subsequent analysis to the E9.5 yolk sac, which contains only HSC-independent hematopoietic lineages. Compared to littermate controls, $PTPN11^{D61Y/+}$; VavCre+ E9.5 yolk sac progenitors demonstrated marked GM-CSF hypersensitivity in colony forming assay at GM-CSF doses between 0.01–1.0 ng/ml ($p<0.05$, n=8). Purified $Ter119^{-}$, $cKit^{+}$, $CD41^{DIM}$ erythro-myeloid progenitors from these samples recapitulated the observed GM-CSF hypersensitivity ($p<0.05$, n=6). Additionally, cultured $PTPN11^{D61Y/+}$; VavCre+ yolk sac progenitors possessed hyperactive Ras-Erk signaling by intracellular flow cytometry using antibodies against pErk and pSTAT5 ($p<0.05$, n=6). We have demonstrated that HSC-independent myeloid lineages from the murine yolk sac possess GM-CSF hypersensitivity and Ras-Erk pathway hyperactivation in a mouse model of JMML. These findings suggest that HSC-independent hematopoietic populations may be involved in the development of JMML *in utero*. They further highlight the need to assess the role of bone marrow independent myeloid lineages in pediatric MPN in order to begin the development of therapeutics that can specifically target these populations.

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Withdrawn

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Acute Alcohol Intoxication Suppresses the *IL-17* Response During Pneumococcal Pneumonia

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Bacterial pneumonia is a complication of ethanol abuse and this susceptibility has been documented in human studies more than a century ago. Excessive ethanol consumption remains a public health problem responsible for approximately 79,000 deaths in the United States each year and for an estimated economic cost of $223.5 billion/year. *Streptococcus pneumoniae (S. pneumoniae)* is the leading etiology of community- acquired pneumonia, especially in older adults and alcoholic patients. Acute ethanol intoxication suppresses the host immune responses against *Streptococcus pneumoniae*. As IL-17 is a critical cytokine in host defense against extracellular pathogens including *S. pneumoniae*, we hypothesized that ethanol impairs mucosal immunity against this pathogen by disrupting IL-17 production and /or IL-17R signaling. A chronic ethanol feeding model in SIV-infected Rhesus macaques and acute ethanol in a murine model were used. Transcriptome analysis of bronchial brushes in the non-human primate model showed downregulation of the expression of IL-17 regulated chemokines in ethanol fed animals, a finding also replicated in the murine model. Surprisingly, recombinant CXCL1 and CXCL5 but not IL-17 or IL-23 + IL1β rescued bacterial burden in the ethanol group to control levels. Taken together, this study suggests that ethanol impairs IL-17 mediated chemokine production in the lung. Thus, exogenous luminal restoration of IL-17 related chemokines, CXCL1 and CXCL5, improves host defenses against *S. pneumoniae*.

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Calorie and Fat Restriction During Chemotherapy Improves Survival in Obese Mice with Acute Lymphoblastic Leukemia

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Obesity promotes the pathogenesis of many cancers, including acute lymphoblastic leukemia (ALL), the most common childhood malignancy. Clinical studies have shown that children who are obese at the time of ALL diagnosis have a greater risk of relapse than normal weight children; however, this risk may be attenuated if their weight normalizes over the majority of treatment. One explanation is that nutrient-deficiency may induce host, but not ALL, cell senescence, thereby augmenting chemotherapy specificity and efficacy. We hypothesize that a calorie- and fat-restricted diet during chemotherapy differentially alters host and ALL cell metabolism, improving chemotherapy efficacy and overall survival of high-fat diet-induced obese mice with ALL. To test the effect of dietary restriction on survival, C57BL/6J mice were placed on a high-fat diet (60% calories from fat) upon weaning. At 20 weeks of age, obese and control mice were implanted with syngeneic BCR/ABL+ ALL cells. After seven days, half of the obese mice were switched onto a low-fat (10% calories from fat) diet. Concurrently, vincristine chemotherapy (0.5mg/kg/wk x 4wks) was initiated and mice were tracked for survival. Separate groups of implanted mice were sacrificed 1 day before, 1 day and 1 week after dietary restriction for bone marrow and spleen collection. BrdU incorporation and p-AKT(Ser473), p-eIF2A(Ser51) and p-S6K(Thr412) levels in marrow and spleen ALL and host cells were analyzed via flow cytometry for changes in cell cycle and metabolism. Finally, we also evaluated the effect of dietary restriction on human ALL outcome, using an obese xenograft ALL model treated with vincristine, dexamethasone and L-asparaginase. Dieted obese C57BL/6J leukemic mice exhibited a greater percentage of weight loss over the first two weeks of chemotherapy compared to non-dieted obese mice (Dieted vs. non-dieted obese: -25.2±10.6% vs. -0.3±4.5%, $p<0.001$). Dieted obese leukemic mice had significantly higher survival than non-dieted obese and lean mice after 4 months (Dieted: 91.6%, Obese: 16.7%, Lean: 41.7%; n=12/group; $p<0.001$). Flow cytometry of the murine bone marrow and spleen showed no diet-induced alterations in BrdU incorporation or AKT pathway activation in either host or ALL cells. Surprisingly, we did not observe a similar effect of dietary restriction on survival in the obese xenograft ALL model. Therefore, a calorie and fat restricted diet initiated at the onset chemotherapy improves overall survival in obese mice with syngeneic ALL, though this phenomenon was not observed in a human xenograft model. Further work is needed to explore the possibility of a dietary intervention as effective potential adjuvant during chemotherapy in obese cancer patients.

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The Role of the MLL-Gene Family in Infant Leukemia and an iPSC Model of Hematopoiesis

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Infant leukemia (IL) is defined as any leukemia diagnosed before 12 months of age. It is a serious disease with a grim prognosis. The incidence of IL is increasing, but epidemiology studies have failed to identify an environmental explanation for this disease (Ross, 2008). Further, several studies in which tumor and normal samples from IL patients were sequenced have shown that there is a paucity of somatic mutation in these tumors. Previously, we have shown that, while there is a lack of somatic mutation in these cases, there is a significant enrichment of rare, non-synonymous germline variation in known leukemia-associated genes, as contained in the Catalog of Somatic Mutations in Cancer (Valentine et al. 2014). This enrichment was seen both in cases of acute myeloid leukemia and acute lymphocytic leukemia, as well as in IL cases with and without an MLL1 translocation, a chromosomal rearrangement that is frequently seen in IL. Interestingly, one of the most variable genes across all of these sub-populations of IL was MLL3, a member of the MLL family of histone-methyltransferases. Further, in cases that lacked an MLL-rearrangement, there was a significant increase of rare, non-synonymous variation in MLL1 and genes that form a complex with it. This enrichment of variation in the MLL1-complex was not observed in IL cases that had a somatically acquired MLL-rearrangement or in healthy controls. This suggests that concurrent dysfunction in both of the functionally distinct MLL1 and MLL3 complexes might be an important contributing factor in IL. To explore the role of the MLL gene family in IL, we have employed an iPSC-based hematopoietic differentiation system (Sturgeon et al, 2014). Using this system we are able to recreate the developmental context of this disease in both IL patient-derived cells as well as healthy controls. We can also modulate the expression of MLL3 using either a CRISPR-Cas knockout approach or an shRNA knockdown strategy. MLL1 function can also be perturbed through the addition of a common MLL-rearrangement to our various iPSC lines. The combinations of patient- and control- derived cell lines with various perturbations of MLL family genes will give us an unprecedented level of insight into the role of these important epigenetic modifiers during early hematopoiesis and possibly leukemogenesis.

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Neurotrophic Factors Selectively Modulate Scratching Behavior and Sensory Neuron Responses to Pruritogens

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Skin biopsies from patients with atopic dermatitis or psoriasis exhibit increased intraepidermal nerve fiber density, increased glial cell line-derived neurotrophic factor (GDNF) and artemin, and increased nerve growth factor (NGF) and its receptor TrkA. Additionally, we have recently demonstrated a selective increase in fibers expressing Ret, the receptor for GDNF family ligands (GFLs), in a mouse model of dry skin itch. These data indicate that neurotrophic factors (NTFs) contribute to the development and maintenance of chronic itch. In our current studies we investigated whether NGF and the GFLs GDNF, neurturin, and artemin can directly induce or modulate histaminergic and non-histaminergic itch. Scratching and wiping behavior after intradermal injection of NTFs into the cheek skin of mice was quantified to determine whether NTFs alone can induce pain or itch. The effects of NTF pretreatment on histamine- and chloroquine-induced scratching were also determined. We found that acutely injected NTFs did not induce scratching or wiping behavior by themselves. On the other hand, pretreatment with NGF significantly and selectively potentiated histamine-induced scratching, while artemin significantly and selectively potentiated chloroquine-induced scratching. To elucidate the mechanisms by which specific NTFs potentiate histaminergic and histamine-independent itch, dissociated trigeminal ganglion neurons were examined for calcium responses to pruritogens and transient receptor potential (TRP) channel agonists. Our results suggest that NTFs selectively increase the proportion of sensory neurons that respond to histamine and non-histaminergic pruritogens.

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Receptor Tyrosine Kinase Signaling Activates Divergent Gene Expression Programs Through Differential Usage of Intracellular Signaling Pathways

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Receptor tyrosine kinases (RTKs) signal through multiple shared intracellular effectors yet mediate many distinct cellular outcomes across a broad range of developmental and oncogenic contexts. To investigate the underlying mechanisms of RTK specificity, we performed RNA-seq to compare the transcriptional response to platelet derived growth factor (PDGF) and fibroblast growth factor (FGF) signaling in primary mouse embryonic palatal mesenchyme (MEPM) cells, as both pathways are required for palate development. While the early gene expression profile induced by both pathways is similar, the late response is divergent. Consistent with this observation, FGF and PDGF treatment have opposite effects on MEPM cell proliferation and differentiation. By comparing the effect of MEK (Mitogen/Extracellular signal-regulated kinase) and PI3K (phosphoinositide 3-kinase) inhibitors on RTK-mediated transcription and cellular outcome, we demonstrate that the FGF response is primarily MEK dependent while the PDGF response is PI3K dependent. Further, pathway inhibition leads to induction of alternate intracellular effectors, thus suggesting one mechanism of compensation in response to inhibitor treatment. Finally, we explore the context specificity of RTK mediated transcriptional programs by comparing our data to published growth factor induced expression profiles in other cell types and signaling patterns during craniofacial development. Collectively, our results identify distinct responses to PDGF and FGF and provide insight into the mechanisms by which transcriptional specificity is encoded into these pathways.

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Increasing Vasculature Through Genetic Down-Regulation or Pharmacological Inhibition of Flt-1 Ameliorates the Dystrophic Phenotype in Duchenne Muscular Dystrophy (DMD) Model Mice

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Duchenne Muscular Dystrophy (DMD) is an X-linked recessive genetic disease in which the gene coding for dystrophin, a membrane-stabilizing protein, is absent. As a result, while the muscle develops normally, it is highly susceptible to contraction-induced injury. The injured muscle is initially repaired by a population of tissue-resident stem cells known as satellite cells. However, continuous rounds of injury lead to an exhaustion of the satellite cell pool resulting in the muscle fiber being replaced by fibrotic scar tissue. Interestingly, these satellite cells are found in close proximity to blood vessel endothelial cells. As such, it is possible that the endothelial cells may create a niche for this stem cell population. While dystrophin is primarily known for its structural support in the skeletal muscle fiber, recent work shows evidence for vascular insufficiency and decreased angiogenesis in the skeletal muscles of DMD patients as well as DMD model (*mdx*) mice. Incidentally, our co-culture experiments show that endothelial cells increase the proliferative capacity of satellite cells. This data indicates that compensating for the defective angiogenic response may result in increased proliferation of satellite cells and improvements of the dystrophic phenotype in mdx mice. Here, we examine mice haploinsufficient for *Flt-1* (VEGF sink trap) crossed with *mdx* mice to produce *mdx:Flt-1+/-* and control *(mdx:Flt-1+/+)* mice. Interestingly, the *mdx:Flt-1+/-* mice showed increased capillary density and an increased number of satellite cells in the muscle. This lead to an amelioration of the dystrophic histopathology and improved muscle function. Lastly, these improvements could be recapitulated by pharmacological intervention in the *mdx* mice using Flt-1-blocking antibodies. These data strongly suggest that increasing the vasculature in DMD can ameliorate the muscular dystrophy phenotype and further validate Flt-1 as a therapeutic target for DMD.

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Net K Secretion in Thick Ascending Limb of Mice on Low Na High K Diet

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Purpose: A low Na and high K diet is considered a healthy choice for its cardiovascular benefits. It is imperative to understand how dietary salt intake influences renal K handling in order to select effective diuretics and prevent hypokalemia and hyperkalemia. **Materials and Methods:** Wild-type (WT) and Ca-activated K channel (BK) β4-subunit knockout mice (KO) were fed either control or a low Na high K diet (LNaHK) for 7-10 days (all groups: N ≥ 4). Urine samples were collected from mice in metabolic cages 12 hours after intra-peritoneal injection of vehicle (polyethylene glycol), furosemide, amiloride, or furosemide + amiloride. High Performance Liquid Chromatography (HPLC) was used to measure the concentration of furosemide in the plasma of furosemide-treated mice. Mouse kidneys were harvested, embedded in paraffin and sectioned onto slides for fluorescent immunohistochemical (IHC) staining with anti-BK-α antibodies. **Results:** In WT on a control diet, the urinary K excretion (UKV, μmol/day) was higher in furosemide-treated mice compared to vehicle due to increased distal flow (furosemide: 390 ± 48 vs vehicle: 297 ± 26). However, in WT on LNaHK, UKV was significantly lower in furosemide-treated mice compared to vehicle despite increased distal flow (furosemide: 609 ± 113 vs vehicle: 1057 ± 121). Furosemide + amiloride treatment decreased UKV more than either drug alone in WT on LNaHK (furosemide + amiloride: 143 ± 41; furosemide: 609 ± 113; amiloride: 413 ± 68). In KO on LNaHK, however, UKV was not different between furosemide and vehicle groups (furosemide: 571 ± 80 vs vehicle: 464 ± 80). Using HPLC, we found the furosemide concentrations in plasma of both WT and KO mice were 2.3 μM and 1.4 μM 2 hours and 6 hours, respectively, after treatment, which would inhibit the basolateral NKCC1 in the collecting ducts by only 20%. Fluorescent IHC staining showed that BK was also expressed in the apical membrane of medullary collecting ducts (MCD) of LNaHK for WT,

but not KO. **Conclusions**: These results suggest that there is a net K secretion in the thick ascending limb of loop of Henle in mice on the LNaHK diet, which is dependent on the BK-mediated K recycling and high interstitial K concentration in the MCD.

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Preclinical TSPO-PET to Image Pancreatic Cancer and High-Risk Precursor Lesions

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Despite improvements in imaging techniques, surgery, and oncologic treatments, clinical outcome for pancreatic ductal adenocarcinoma (PDAC) has not improved in 40 years. To improve outcomes, it is imperative to develop new imaging capabilities that enable early detection of PDAC and to identify pancreatic cancer precursor lesions that are likely to progress to invasive cancer. Pancreatic intraepithelial neoplasia (PanIN) and intraductal papillary mucinous neoplasia (IPMN) represent two important classes of PDAC precursor lesions; risk stratification among these lesions represents a unique opportunity for personalized medicine in patients at risk for developing PDAC. We identified translocator protein (TSPO), an 18 kDa outer mitochondrial membrane protein involved in cholesterol metabolism,as a potential biomarker of pancreatic cancer. We evaluated TSPO immunoreactivity (IHC) in tissue microarrays (TMAs) of human PanINs, IPMNs, and PDAC and correlated TSPO levels with the degree of dysplasia, mutational status, and lesion subtype. TSPO IHC was elevated in high-grade human PanIN lesions and high-grade subtypes of IPMN compared to low-grade PanIN and benign IPMN subtypes. These results prompted our evaluation of TSPO-PET in preclinical pancreatic cancer and exploration of the mechanistic basis of elevated TSPO levels in this setting. We evaluated ^{18}F-VUIIS-1008, a novel TSPO PET ligand, in genetically engineered mouse (GEM) models of PanIN to PDAC progression. Robust uptake of ^{18}F-VUIIS-1008 and correspondingly increased TSPO IHC was observed in high-grade PanINs and PDACs arising in Pft1a-Cre/+; LSL- Kras^{G12D}/+, Tgfbr2^{fl}/+ mice. In contrast, only modest TSPO PET and TSPO levels were observed in PanINs arising in Pft1a-Cre/+; LSL- Kras^{G12D}/+ mice, which rarely progress to PDAC. These data suggest that TSPO PET is a promising, non-invasive modality to image PDAC and to identify high-risk PDAC precursor lesions. Ongoing studies are now evaluating TSPO PET in GEM models of IPMN to PDAC progression. We anticipate that these studies will lay the foundation for a trial evaluating TSPO PET in patients at high-risk for developing pancreatic cancer.

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The Use of Team Learning Activities to Maintain Clinical Competencies and Foster New Clinical Knowledge in Dual Degree MD/PhD Students

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The majority of students in combined MD/PhD programs take a leave of absence from medical training between the second and third years of medical school to complete a PhD degree. This extended period away from clinical activity can be detrimental to both clinical skills and clinical knowledge, placing dual degree students at a disadvantage when they return to clinical clerkships and increasing student anxiety relating to this transition point. In addition, MD/PhD students have fewer elective rotations during the third and fourth years of medical school, limiting opportunities to explore the variety of medical specialties available for residency or fellowship training. We have developed a Continuing Clinical Education course for MD/PhD students in graduate school to maintain clinical skills and knowledge acquired during the first two years of medical school and to increase exposure to different medical specialties. The course features a three-pronged curriculum of clinical encounters, shadowing experiences, and clinical knowledge sessions. The effectiveness of the course is being measured through analysis of academic data including clerkship grades and exam scores for 1) MD/PhD students who completed the course, 2) MD/PhD students who returned to clinic prior to inception of the course, 3) MD students who took a leave of absence for one year or more of research, and 4) MD students who did not take a leave of absence. In addition, a longitudinal repeated-measures survey is being provided to all MD/PhD students enrolled in the course to assess clinical readiness and to track changes in anticipated choice of medical specialty. Data comparisons between experimental groups will reveal the impact of 1) academic leave of absence and 2) the Continuing Clinical Education course on performance in clinical clerkships. These results will be used to determine the importance of clinical continuity in MD/PhD training programs.

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MAC in Immunocompetent

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Disseminated mycobacterium avium complex (MAC) is historically seen in immunodeficient patients. The infrequency of encounters in immunocompetent people lessens its likelihood to be included in the differential. A 56 year old male presented to the emergency department with night sweats, cold intolerance, diarrhea, low grade fever (100.4°F) and productive cough for 4 days. His past medical history is significant for duodenal stricture, osteoarthritis, chronic low back pain, recurrent kidney stones, and GERD. His past surgical history includes EGD with duodenal dilatation, ureteroscopy, spinal fusion, removal of spinal hardware, laparoscopic cholecystectomy, and appendectomy. He was previously employed as a city bus driver and lifelong non-smoker. His physical exam was normal. On admission, CT-Abdomen was negative for any evidence of colitis or other acute processes but incidentally showed left lower lung nodule. He had an elevated WBC count that returned to normal over 2 days and an increased CRP. Three days later, a CT of chest revealed that the nodules had increased in size compared to his 2012 examination. Additionally, there was mediastinal and bilateral hilar adenopathy. He had a slightly elevated ACE level of 66, which is not high enough to be sarcoidosis. Bronchoscopy was performed revealing purulent drainage. Biopsy showed caseating granulomas from his subcarinal lymph node and lung nodule. Gram stain, AFB staining, and special staining revealed no organisms. This biopsy did not show any evidence of malignancy. Aerobic culture of bronchoalveolar lavage grew heavy Group A and B streptococcus species. His histoplasma, legionella, aspergillus, and tuberculosis antigens were negative from his lung biopsy. He was discharged on Augmentin but then readmitted due to worsening diarrhea and continued to have night sweats and chills. He met SIRS criteria with a WBC count of 14, fever, and tachycardia. He was found to be negative for C. difficile. The patient completed his 10-day course of Augmentin from his first discharge. Finally, his mycobacterium growth indicator tube (MGIT) from initial bronchoscopy samples grew MAC. He was prescribed a 12 months of rifampin, azithromycin, and ethambutol and one month later showed significant clinical improvement. This case highlighted the importance of establishing a broad differential diagnosis for all cases in order to expedite patient diagnosis and treatment. MAC is well recognized as an infection of immunocompromised and is relatively rare in immunocompetent patients. Due to this fact, MAC was found after a month of incubation yielded growth. Blood cultures from admission were negative and thus could represent important data concerning dissemination that remained untapped.

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Disruption of Hepatocellular ERBB3 Expression Suppresses Diethylnitrosamine-Induced Hepatocellular Carcinogenesis

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The liver has an extraordinary ability to regenerate in response to injury. With chronic injury, regeneration can eventually become dysregulated, leading to hepatocellular carcinogenesis. Hepatocellular carcinoma (HCC) is the second leading cause of cancer death with ineffective treatment options and a five-year survival rate of 12%. Members of the ERBB receptor tyrosine kinase family are major regulators of liver regeneration and may have a role in HCC. To test the hypothesis that ERBB3 and EGFR (ERBB1) are important regulators of HCC formation, ERBB3 and EGFR were inactivated in a hepatocellular-specific manner by breeding Cre-recombinase and ERBB3 or EGFR loxP mice. To initiate tumorigenesis, diethylnitrosamine (DEN) was injected into wildtype (WT), ERBB3 hepatocyte-specific knockout (HS-KO) and EGFR HS-KO mouse pups. At 8 months, mouse liver tissue was harvested, tumors were counted and cell proliferation was assessed. It was determined that ERBB3 HS-KO mice, but not EGFR HS-KO mice, had a reduction in hepatic tumors ($p<0.01$) and lower levels of the two proliferation markers that were evaluated, Ki67 ($p=0.05$) and cyclin A ($p<0.01$). ERBB2, normally not expressed in adult mouse liver, was detected in DEN-injected mice. ERBB3 HS-KO mice showed reduced ERBB2 expression ($p<0.01$), suggesting that the induction of ERBB2 may be partly dependent upon ERBB3. ERBB3 HS-KO resulted in diminished STAT3 activation ($p<0.01$), but did not affect activation of other downstream signaling molecules AKT, ERK1, or ERK2. These observations suggest that ERBB3 may have a role in hepatocellular carcinogenesis and could become a target in the treatment of HCC.

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CD103+ CD11b+ DCs Promote Commensal-Specific Th17 Responses Independently of Cytokine Polarization or Antigen Presentation

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The classical paradigm of dendritic cell (DC) function is based upon the activation and migration of antigen-bearing DCs from mucosal and epithelial barriers to draining lymph nodes that are rich in naïve lymphocytes. Mounting evidence shows that specific dendritic cell subsets are required to specify naïve helper T cell differentiation to the Th1, Th2, or Th17 effector lineages. However, the mechanisms whereby distinct DC subsets promote the acquisition or maintenance of T helper cell identity remain incompletely understood. Here we use murine transgenic and commensal-specific reagents to show that intestinal CD103+ CD11b+ DCs are required for Th17 responses induced by colonization with segmented filamentous bacteria (SFB) under homeostatic conditions. However, LP Th17 homeostasis was

preserved in mice with subset-restricted ablation of canonical Th17 polarizing factors, including IL-6, IL-1β, IL-23, and integrin β8. SFB-specific T cells underwent normal proliferation and expansion in the absence of CD103+ CD11b+ DCs, confirming that these DCs are not required to present commensal antigen. However, the Th17 identity of SFB-specific cells was not maintained in the absence of CD103+ CD11b+ DCs. This unexpected finding suggests that non-paradigmatic functions of dendritic cells are essential for host-commensal immune fitness.

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Circadian Timing of Skeletal Muscle Gene Expression and Biological Processes

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Circadian rhythms are 24-hour cycles of behavior and physiology, including skeletal muscle physiology. The current study uses computational methods and leverages the large amount of publicly available microarray data in order to elucidate the circadian transcriptional program in skeletal muscle. The purpose of this study is to gain insight into the timing of genetic control in relationship to known circadian timings for skeletal muscle physiology. Analyses are performed on high-resolution mRNA time-course data (GSE54652) collected from the gastrocnemius muscle in the wild-type mouse hind-limb every 2 hours for 48 hours. Roughly 1600 genes with circadian expression patterns (*Adj. $p < 0.05$*) are identified in skeletal muscle using the non-parametric JTK-Cycle algorithm. Kolmogorov-Smirnov (KS) test of Gene Ontology terms shows that the most significantly enriched biological processes for these circadian genes include response to stimulus (10^{-15}), immune system processes (10^{-15}), muscle and vascular development (10^{-13}), and metabolism and/or the regulation of metabolic processes (10^{-15}), all of which are vital to skeletal muscle tissue maintenance. All circadian genes are clustered using an algorithm that only uses mutual information and makes no prior assumptions (Iclust) and subsequently analyzed for gene ontology enrichment using the iPAGE package. Results show that the up-regulation of circadian genes involved in the same pathways occurs roughly at the same time of day, and the timing of peak gene expression appears to precede the skeletal muscle physiological process associated with that particular pathway. In conclusion, skeletal muscle appears to tightly regulate the daily timing of gene expression to ensure the correct and timely occurrence of key cellular processes. Mismatch between skeletal muscle physiological demand and genetic timing may result in suboptimal physical performance and tissue health, which may imply gradual but long-term deficits. These results further highlight the molecular etiology of circadian disruption on skeletal muscle health.

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Differential Reliance on Autophagy for Protection from HSV Encephalitis Between Newborns and Adults

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Newborns are more susceptible to severe disease from infection than adults, with maturation of immune responses implicated as a major factor. The type I interferon response delays mortality and limits viral replication in adult mice in a model of herpes simplex virus (HSV) encephalitis. We found that intact type I interferon signaling did not control HSV disease in the neonatal brain. However, the multifunctional HSV protein γ34.5 involved in countering type I interferon responses was important for virulence in the brain in both age groups. To investigate this observation further, we studied a specific function of γ34.5 which contributes to HSV pathogenesis in the adult brain, inhibition of the cellular process of autophagy. Surprisingly, we found that the beclin binding domain of γ34.5 responsible for inhibiting autophagy was dispensable for HSV disease in the neonatal brain, as infection of newborns with the deletion mutant decreased time to mortality compared to the rescue virus. Additionally, a functional beclin binding domain in HSV γ34.5 did not effectively inhibit autophagy in the neonate, unlike in the adult. Type I IFN responses promote autophagy in adult, a finding we confirmed in the adult brain after HSV infection; however, in the newborn brain we observed that autophagy was activated through a type I IFN-independent mechanism. Furthermore, autophagy in the wild-type neonatal mouse was associated with increased apoptosis in infected regions of the brain. Observations in the mouse model were consistent with those in a human case of neonatal HSV encephalitis. Our findings reveal age-dependent differences in autophagy for protection from HSV encephalitis, indicating developmental differences in induction and regulation of this innate defense mechanism after HSV infection in the neonatal brain.

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PepO, a CovRS-Regulated Aminopeptidase, Counteracts *Streptococcus pyogenes* Quorum Sensing

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Group A Streptococcus (GAS, Streptococcus pyogenes) is a major cause of human disease, resulting in over 600 million cases of streptococcal pharyngitis per year. GAS has many phenotypic states, ranging from a cause of pharyngitis to systemic and invasive diseases. It has been shown that GAS exists in the body in at least two different lifestyles: in an avirulent mode of carriage or in a virulent disease-causing state. The mechanism by which GAS maintains these lifestyles is unclear, but the ability to coordinate gene expression across bacterial populations undoubtedly plays an important role. Our lab has identified a novel GAS intercellular communication network, also known as a quorum-sensing (QS) network, which appears to induce biofilm development and immune system defenses. GAS cell-to-cell communication utilizes short hydrophobic peptides (SHPs) that are produced by the bacteria, are secreted across the membrane, and are processed into mature signaling pheromones capable of dispersion among members of the community. SHPs are imported into the cell and upon reaching critical concentrations within the cytosol bind to their cognate Rgg receptors that serve as transcription factors to control target-gene expression. Activation of at least one identified operon leads to biofilm formation, increased lysozyme resistance and may improve adherence in a tissue culture colonization model. Despite the advances we have made in understanding this QS system, we do not have a clear understanding of how QS in GAS impacts human health. Genetic studies have identified a link between Rgg QS and the Control of Virulence (CovRS) pathway, a central controller of GAS virulence, via the aminopeptidase PepO. We hypothesize that *pepO* is regulated by CovRS and alters Rgg2/3 induction by degrading SHP pheromones. Consistent with this hypothesis is the finding that *pepO* mutants show sustained SHP activities in culture supernatants, while strains expressing *pepO* on a multi-copy plasmid from a constitutive promoter (presumably over-producing PepO) demonstrate attenuated responses to exogenous SHP. This putative regulatory link between the Rgg2/3 QS circuit and the CovRS system supports the notion that induction of the Rgg2/3 quorum-sensing network contributes to the community-wide behavior of asymptomatic carriage.

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Glucagon Stimulation Test to Diagnose Growth Hormone Deficiency: A Single Institution's Experience

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Background: The use of the glucagon stimulation test (GST) to diagnose growth hormone deficiency (GHD) in adults with pituitary disease is increasingly common as alternative methods have become unavailable or more cumbersome. A recent study found that body mass index (BMI) and response to oral glucose tolerance testing (OGTT) negatively correlated with peak GH in healthy overweight men (Dichtel L, JCEM 2014). The goal of this retrospective study was to investigate the effect of BMI and gender on GH response among patients with pituitary disease. We secondarily examined the relation between blood glucose (BG) and GH dynamics following glucagon. **Methods:** Adult patients from September 2009- 2014 who underwent GST at Vanderbilt Medical Center were included. Continuous variable comparisons between groups were analyzed using the Mann-Whitney U-test. Spearman correlation was used to determine an association between continuous variables. Results are presented as mean ± standard deviation unless otherwise noted. **Results:** 42 patients (N=28; 66.7% female) had sufficient data available for analysis. The mean age of patients was 39.4$\pm$13.4 years and BMI was 34.3$\pm$8.4 kg/m^2; this did not differ by gender. Peak GH in females was not significantly higher than in males (3.10$\pm$3.78 ng/mL vs. 2.17$\pm$5.23; p=0.17). Females prescribed oral estrogen (N=7) trended towards higher peak GH response (p=0.06). We did not find a linear association between BMI and peak GH. Obese men (N=7) did not stimulate lower than men with BMI <30 kg/m^2 (p=0.52). Obese women (N=19), however, stimulated lower than women with BMI <30 kg/m^2 (peak GH 2.02 ±2.80 ng/mL vs. 5.39 ±4.68; p=0.03). Obese women also had a higher nadir BG (63.9±13.1 mg/dL vs. 52.7 ± 9.9; p=0.03). Extent (ΔBG) and rate of change in BG during GST did not correlate with peak GH. Though the number of anterior pituitary hormone deficiencies inversely correlated with peak GH (r_s=-0.38; p=0.01), obese women did not have significantly more anterior pituitary hormone deficiencies than non-obese women (p=0.44). **Conclusions:** Obese women with pituitary disease do not respond as robustly to GST, and may be misclassified as GHD with the current cut-off of peak GH response <3 ng/mL. This may be secondary to altered glycemic response to glucagon, as suggested by higher BG during GST. These results support the recommendation to consider a lower GH cutoff for diagnosis in obese individuals. Alternatively, GH cutoff may need to be adjusted to nadir BG obtained during GST. Further studies investigating the validity of the GST in patients with impaired glucose tolerance and diabetes mellitus are needed.

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Inhibition of the Epidermal Growth Factor Receptor Attenuates Aortic Aneurysm Formation

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Aneurysm formation is the phenotypic manifestation of progressive organ failure of the large arteries. Within the aorta, aneurysms occur in the thoracic aorta as well as the abdominal aorta. Although pathologically distinct, in murine models both abdominal and thoracic aortic aneurysms (AAA and TAA, respectively) have been shown to be dependent upon angiotensin II (angII) signaling via the angiotensin type I receptor (AT1R). AT1R-mediated aortic aneurysm formation requires activation of mitogen-activated protein kinases such as extracellular-regulated kinase (ERK) 1/2 with subsequent increases in pro-aneurysmal mediators such as matrix metalloproteinases -2 and -9. AT1R-mediated ERK acrivation has also been shown *in vitro* to require transactivation of the epidermal growth factor receptor (EGFR). Based on this, we hypothesized pharmacologic inhibition of the EGFR would attenuate AAA and TAA development by inhibiting this AT1R-ERK signaling pathway. To test this hypothesis, we utilized a previously established murine model of AAA and TAA formation in which we infused hyperlipidemic *ApoE*$^{-/-}$ mice with angII for 4 weeks. We followed abdominal and thoracic aortic dilation with transabdominal and transthoracic echocardiography, respectively. We observed treatment with the EGFR inhibitor AG1478 resulted in significant attenuation of both AngII-induced AAA and TAA formation. Additionally we found activation of ERK as well as production of MMP-2 and -9, was significantly reduced within aneurysmal tissue harvested from these animals. To confirm these findings, we stimulated primary thoracic aortic vascular smooth muscle cells (VSMC) with angII in the absence or presence of AG1478. Consistent with our *in vivo* findings, blockade of the EGFR resulted in inhibition of angII-induced ERK 1/2 activation as well as angII-induced MMP-2 and -9 production in primary VSMCs.These results suggest the EGFR mediates AT1R-mediated aneurysm formation by regulating AT1R-mediated ERK 1/2 activation. We conclude, therefore, the EGFR may serve as a novel target for inhibition in the prevention and treatment of aortic aneurysms.

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Withdrawn

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Effects of *rpd3* Mutation on Mitochondrial Function and Metabolism in Aging *Drosophila*

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Previously our lab showed that mutations in *rpd3* (*Drosophila HDAC1* homologue) extend the lifespan of *Drosophila* through a mechanism that overlaps with caloric restriction (CR). CR is known to increase lifespan by altering many physiological processes, including mitochondrial function and nutrient metabolism. The objective of this project is to determine the mechanism of lifespan extension in heterozygous *rpd3*-mutant flies as it relates to mitochondrial function, stress response, and metabolic homeostasis. The mitochondrial-to-nuclear gene ratio, level of *spargel* (*Drosophila PGC-1* homologue) mRNA, and quantification of electron micrographs were examined as indicators of mitochondrial biogenesis and quantity. Mitochondrial respiration was examined to analyze differences in mitochondrial function. qPCR and mRNA sequencing were used to examine changes in genes responsible for metabolism, as well as other cellular pathways that affect longevity. We have found significant differences in mitochondrial respiration in fruit flies with heterozygous mutations of *rpd3*. However, our results indicate no difference in mitochondrial biogenesis. Many genes of the insulin-signaling pathway are differentially expressed in *rpd3*-mutant flies. Based on these results, we conclude differences in mitochondrial biogenesis are not the reason for lifespan extension in *rpd3*-mutant flies as initially hypothesized. The insulin-signaling pathway remains a candidate pathway for the lifespan extending effects. Future work will be needed to determine how targeting this pathway could promote healthy aging and lifespan extension.

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Identifying the Key Immunological Parameters that Correlate with Better Survival of Patients with Pancreatic Cancer Receiving a Pancreatic Tumor Cell-based Vaccine

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Background: Pancreatic ductal adenocarcinoma (PDAC) is one of the most dreadful diseases and considered a "nonimmunogenic" neoplasm. Single-agent immunotherapies have failed to demonstrate significant clinical activity in PDAC and other "nonimmunogenic" tumors, in part due to a complex tumor microenvironment (TME) that provides a formidable barrier to immune infiltration and function. We designed a neoadjuvant and adjuvant clinical trial comparing an irradiated, granulocyte-macrophage colony-stimulating factor (GM-CSF)–secreting, allogeneic PDAC vaccine (GVAX) given as a single agent or in combination with low-dose cyclophosphamide to deplete regulatory T cells (Treg) as a means to study how the TME is altered by immunotherapy. **Experimental design:** Examination of resected PDACs revealed the formation of vaccine-induced intratumoral tertiary lymphoid aggregates in 33 of 39 patients 2 weeks after vaccine treatment. Lymphoid aggregates were micro dissected from pancreatic tumor tissue. Then, we isolated RNA from these tumor lymphoid aggregates, performed an expression microarray for the lymphoid aggregates' functional profile, and conducted Gene Set Enrichment Analysis to uncover upregulated and downregulated genes. Our microarray findings were validated with quantitative Real Time-PCR of mRNA transcripts from tumor infiltrating lymphocytes taken from fresh PDAC tissue, testing 5-6 genes from each pathway (Th1, Th2, Th17, and Treg). Furthermore, we performed immunohistochemistry to characterize the expression of these genes and other genes involved in regulating the immune response, including PD-L1, on tumor samples from vaccinated patients. **Results:** Microarray analysis of microdissected aggregates identified gene-expression signatures in five signaling pathways involved in regulating immune-cell activation and trafficking that were associated with improved post-vaccination responses. A suppressed Treg pathway and an enhanced Th17 pathway within these aggregates were associated with improved survival, enhanced postvaccination mesothelin-specific T-cell responses, and increased intratumoral Teff:Treg ratios. Immunohistochemical analysis showed these aggregates to be regulatory structures of adaptive immunity. Post-GVAX T-cell infiltration and aggregate formation resulted in the upregulation of immunosuppressive regulatory mechanisms, including the PD-1–PD-L1 pathway, in the lymphoid aggregates in tumors from vaccinated patients. **Conclusion:** This study provides the first example of immune-based therapy converting a "nonimmunogenic" neoplasm into an "immunogenic" neoplasm by inducing infiltration of T cells and development of tertiary lymphoid structures in the TME. In addition, upregulation of immunosuppressive regulatory mechanisms suggest that patients with vaccine-primed PDAC may be better candidates than vaccine-naïve patients for immune checkpoint and other immunomodulatory therapies.

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Disease Modeling and Physiological Drug Screening for Familial Dilated Cardiomyopathy Using Human Induced Pluripotent Stem Cells

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Dilated cardiomyopathy (DCM) is a leading cause of heart failure. Despite heritability of DCM, development of heart failure is unpredictable into adulthood, suggesting an insidious degenerative process. Recently, genetic linkage analysis in families with autosomal-dominant DCM identified a novel heterozygous missense mutation in RNA binding motif protein 20 (*RBM20*). We hypothesized that *RBM20* is an essential component of the RNA processing machinery during cardiogenesis and is required to regulate cardiac gene expression to pattern normal structural and physiological integrity of newly formed cardiomyocytes (CMs). Induced pluripotent stem cells (iPSCs) were generated from unrelated patients with identical missense mutation. *RBM20* familial DCM-specific iPSCs had the hallmarks of pluripotency as shown by immunostaining, teratoma formation, and karyotyping. *RBM20* familial DCM iPSC-CM structural integrity exhibited atypical sarcomeric geometry. Similarly, *RBM20* iPSC-CM functional maturity showed defective calcium handling machinery. Effect of chronotropic stress was also analyzed with 10μM norepinephrine, demonstrating susceptibility in *RBM20* iPSC-CMs. To probe DCM development and unmask the initial molecular dysfunction, stage-specific *RBM20* transcriptome was evaluated by microarray, identifying differentially expressed genes linked to known cardiac pathology ranging from angiogenesis regulator *CYP26B1* to embryonic heart development factor *TBX18* as the initial molecular aberrations. Modeling human cardiac disease according to a stage-specific cardiogenic roadmap allowed us to establish a new paradigm of familial DCM pathogenesis as a developmental disorder that is patterned during early cardiogenesis and propagated with cellular mechanisms of pathological cardiac remodeling. This model was incorporated into successive levels of a screening platform using the xCELLigence RTCA Cardio system to identify drugs that preserve cardiomyocyte phenotype and calcium handling properties *in vitro* during chronotropic stress. In this work, we present a patient-specific iPSC model of a complex genetic condition, showing the power of this technique for discovery and testing of therapeutic strategies for a heart disease with important clinical significance.

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Radiosensitization of Glioblastoma Stem Cells by Targeting High-Affinity Glucose Uptake

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Purpose: Glioblastoma (GBM) is a deadly form of brain tumor for which conventional treatments including radiation therapy are not curative. Glioblastoma stem cells (GSCs) are a small population of the tumor that is responsible for tumor maintenance and resistance to therapies. We showed that GSCs survive conditions with restricted nutrients by using a mechanism of high-affinity glucose uptake from normal nerve cells to outcompete for nutrients. In this study, we aim to understand how high-affinity glucose transporter (Glut3) promotes GBM radioresistance through maintaining tumorigenic hierarchical growth pattern, and to evaluate the therapeutic efficacy of inhibiting Glut3 to sensitize GSCs to radiation therapy. **Methods:** 1. Examine the functional importance of Glut3 in tumorigenesis through xenograft mouse model. GSCs expressing Glut3 shRNA were implanted intracranially into immunocompromised mice. Tumor incidence, volume, and mice median survival were recorded. 2. Define the therapeutic benefits of down-regulating Glut3 on GSCs in conjunction with radiation. Glut3-targeting and control shRNAs were given to patient-derived GSCs in combination with radiation (3Gy). Cell survival assay was conducted 3 days after radiation to evaluate the effect of Glut3 knockdown. 3. Characterize the expression level of Glut3 in determining GBM patient survival. Based on TCGA (The Cancer Genome Atlas) database, the predictive value of Glut3 for GBM patient outcome was evaluated by examining the correlation between Glut3 expression level and overall survival. **Results:** 1. Glut3 is required for in vivo tumorigenesis of GSCs. In vivo tumor propagation assay with patient-derived GSCs demonstrated that targeting Glut3 using shRNA increased the survival of mice bearing human GBM xenografts relative to non-targeting shRNA ($p<0.0004$). This result demonstrated the essential role of Glut3 in maintaining GSC function in vivo. 2. Knocking down Glut3 sensitizes GSCs to radiation. Glut3 knockdown significantly decreased GSC survival after radiation compared to control (1.7 fold). This result revealed the function of Glut3 in promoting GSC survival after radiation therapy, consistent with its key role in GSC maintenance. 3. Glut3 expression level correlates with GBM patient survival. To evaluate the role of Glut3 in determining GBM patient survival, we generated Kaplan-Meier survival curves using TCGA dataset. Glut3 expression informed poor prognosis, whereas other glucose transporters (Glut1/2/4) did not correlate with patient outcome. **Conclusion:** Our results demonstrated that Glut3 plays a key role in enforcing GBM cellular hierarchy and promoting radiation resistance. This study provided scientific rationale to apply anti-glucose metabolism medication as potential adjuvant therapy to eradicate GBM and to develop imaging tools to detect GSCs in patients.

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Diagnosis of Prostate Cancer Using Multiparametric Prostate Magnetic Resonance Imaging and Ultrasound Fusion Biopsy for the Cohort of Men with Initially Negative Transrectal Ultrasound Biopsy

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Introduction and Objectives: Multiparametric MRI and ultrasound (mpMRI/US) fusion target biopsy may be a potentially beneficial imaging tool in detecting prostate cancer in individuals with prior negative transrectal ultrasound (TRUS) biopsies. In addition, PSA density calculated using MRI based prostate volumes has significant prognostic benefit and may reduce unnecessary repeat biopsies. **Methods:** A retrospective review was performed on consecutive patients with history of mpMRI at Loyola from 2013 to 2014. All patients with previous negative 12 core TRUS biopsies prior to presentation to Loyola were included. Patients underwent endorectal coil mpMRI, and those with lesions identified proceeded to mpMRI/US fusion target biopsy. Demographic and pathologic data were collected and analyzed.**Results:** We identified 45 patients with history of prior negative biopsies. 5 (13.2%) patients had no areas of suspicion based on mpMRI and were able to forgo further biopsy. Of the 40 patients in whom mpMRI detected suspicious lesion, MRI/US fusion target biopsy identified cancer in 12 patients (30%). Test sensitivity was 100%, specificity 15%, and negative predictive value 100%. 2 patients had high risk cancer (gleason score 9 and 8, respectively), and 4 were in intermediate risk (gleason score 7). Cancer positive group had overall higher mean PSA (9.7 [3.52-16.7]), PSAD (0.181 [0.087-0.471]), and prostate volume (59.4 [35.4-104.8]) compared to cancer negative group. Univariate analysis revealed statistically significant difference in age and PSA between cancer positive and cancer negative groups. Those with positive targeted biopsies were older (median age of 67 compared to 60.5;$p=0.04$) and had higher PSA values (median value of 9.4 compared to 5.3; $p=0.03$) than those with negative targeted biopsies. **Conclusions:** mpMRI in conjunction with MRI/US UroNav fusion biopsy platform is a valuable diagnostic tool for detecting prostate cancer in individuals with initially negative TRUS biopsies. MRI and MRI/US fusion biopsy upstaged 30% of cases with fewer cores, limiting the number of iterative biopsies. PSA density based on MRI prostate volume calculation were also higher in cancer positive patients. Further study is warranted.

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Minimal Residual Disease Testing in AML Using Error-Corrected Sequencing

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Following treatment for acute myeloid leukemia (AML), the accurate assessment of residual disease is essential for risk stratification and outcome prognostication. Currently, minimal residual disease (MRD) testing is accomplished using either multiparameter flow cytometry (MPFC), which targets clonal cell surface markers, or qPCR, which targets leukemia-associated chromosomal translocations. However, these methods are useful in only a subset of leukemia patients. Conversely, virtually every case of AML is marked by specific somatic mutations within a set of several recurrently mutated genes (Cancer Genome Research Atlas Network, *NEJM* 2013; Radtke I, et al., *PNAS* 2009). These leukemia-specific mutations present a potential target for MRD testing. While the specific constellation of mutations present within the leukemia will differ from person to person, the mutations largely occur within a discrete set of genes recurrently mutated in AML. A decade of research utilizing next-generation sequencing (NGS) technology has identified these genes and methods already exist to target these regions for sequencing. Despite these advancements, the direct application of NGS to MRD testing is precluded by the approximately 1% error rate of the sequencing technology. For comparison, MPFC and qPCR provide prognostic information to a detection limit of 1:10,000 cells (Borowitz MJ, et al., *Blood 2008)*. Fortunately, methods have been developed for error-corrected sequencing (ECS), which enable the reliable detection of rare variants to a similar threshold (Kinde I, et al., *PNAS* 2011; Schmitt MW, et al., *PNAS* 2012). Already, we have applied these methods to study the clonal evolution of rare *TP53* mutations during the early stages of leukemogenesis (Wong TN, et al. *Nature* 2014; Young AL, et al., *under review*). Here, we present the application of ECS as a NGS-based MRD platform that targets leukemia-associated somatic mutations in 54 genes recurrently mutated in AML. This enabled us to quantify residual clones harboring leukemia-associated mutations to a threshold of 1:10,000 cells. We benchmarked these methods with remission samples obtained from 15 patients treated for AML and directly compared ECS-MRD to conventional MRD. This technology has significant translational potential as a universal MRD testing platform. This method will improve outcome prognostication by enhancing the accuracy of residual disease quantification following treatment. Furthermore, this method offers insight into the biology of AML by describing the clonal architecture of a patient's leukemia at the genomic level. Combining this information with the growing arsenal of targeted therapeutics will facilitate the personalization of treatment and potentially improve clinical outcomes in these patients.

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HSV Esophagitis in an Immunocompetent Teenager

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Herpes esophagitis is a common infection in the immunocompromised host. However, it is a rare condition in the immunocompetent population, and usually presents with the constellation of symptoms of odynophagia, fever and retrosternal chest pain. In immunocompetent patients, symptoms of odynophagia and chest pains are usually attributed more to pill induced esophagitis, toxic ingestion or reflux esophagitis. Rarely, if ever, is infectious esophagitis from HSV, CMV or candida considered. HSV-1 is more commonly the cause of esphoagitis, but typically as a reactivation. In very rare cases does it present as a primary infection in the esophagus. We describe a case of HSV esophagitis in an eighteen year old immunocompetent host with no significant past medical history. He initially presented to his primary care physician with complaints of odynophagia and was prescribed a course of amoxicillin and prednisone syrup. He presented four days later to the emergency room with worsening odynophagia, retrosternal chest pain and anorexia. He was evaluated by the gastroenterology team and was taken for esphsophagogastroduodenoscopy (EGD), which revealed diffuse bleeding superficial ulcerations along the entirety of his esophagus. Biopsies were taken and subsequently found to be HSV positive. The patient was treated with intravenous acyclovir, proton pump inhibitor, and sucralfate. HIV status on the patient was found to be negative, and no other causes of immunosuppression was found. We believe the patients' initial presentation was in fact, HSV esophagitis, which was exacerbated by his prednisone use. The purpose of this report is to highlight the rare occurrence of infectious esophagitis in otherwise healthy, young individuals and to be able to promptly diagnose and begin treatment on such patients. EGD with biopsy is the gold standard diagnostic modality for HSV esophagitis and should be considered in young patients who present with odynophagia with or without other alarm features. EGD findings can point to the cause as HSV as it has very typical appearance on EGD. Often we see superficial, well-demarcated ulcers, typically along the mid to distal esophagus. The treatment of choice for HSV esophagitis is intravenous acyclovir 5mg per kg every eight hours for seven to fourteen days. Treatment should be initiated promptly following the EGD to expedite resolution. Symptoms for most immunocompetent patients resolve spontaneously in about one to two weeks. Although rare, HSV esophagitis should be entertained given the correct constellation of history and symptoms. In confirmed cases of HSV esophagitis, it is important to reassess the patient for any underlying immunodeficiencies.

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Preventive Care: How Comorbidities Affect Screening Rates

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Many studies have concluded that beneficial preventive care is under-utilized. However, little is known about how utilization varies across different comorbid groups. Comorbid patients could have less utilization because they have a lower life expectancy, decreasing the gains from long-term prevention. Alternatively, comorbid populations could place greater value on prevention because existing comorbidities often complicate the treatment of new conditions. Additionally, comorbid patients are more likely to interact with physicians, which makes them more likely to be offered (and receive) preventive services. Overall, it is not obvious whether the screening rates should be expected to be higher or lower than that of the healthy population. Using the 2001-2009 Medical Expenditure Panel Survey, baseline results show that PSA and mammography rates vary significantly across different comorbid groups. There are significantly higher screening rates among those with generalist-managed comorbidities (e.g. high blood pressure, high cholesterol), but lower rates among those who require more specialist care (e.g. previous stroke, heart attack). Since these comorbidities have similar negative effects on life expectancy, traditional economic models would have predicted that they also have similar effects on screening rates. It is possible that differences in physician visit patterns could be an intervening factor. Count models of annual visits confirm that there are differences in physician utilization rates across comorbid groups. Generalist-managed conditions are correlated with more visits on all margins while specialist-managed conditions had no correlation with visits. The association between visits and screening rates is difficult to empirically control for because these variables are inherently simultaneously determined: visits increase the likelihood of screening but screening tests necessarily require visits. This simultaneity would likely result in a positive bias of the estimated correlation between visits and screening. Regression results show that controlling for physician utilization halved the estimates for generalist-managed conditions, but there was no change in the estimates for specialist-managed conditions. Like the count model results, these results also suggest that these comorbid groups might have different patterns of physician utilization. Taken together, patients with generalist-managed conditions have higher screening rates, partly due to more visits, but patients with specialist-managed conditions have lower screening rates, with no change in physician visits. Overall, the differences in screening rates between different comorbid populations suggest that efforts to improve health and/or contain cost by increasing preventive care will need to consider the varying determinants of demand for screening across different comorbid populations.

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KLF15 Establishes the Landscape of Circadian Expression in the Heart

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Circadian rhythm is critical for maintaining cardiovascular health; its importance is underscored by increased cardiovascular disease in both human shift workers and animal models with disrupted core clock function. The core molecular clock functions in all tissue and cell types and regulates gene expression. However, how each tissue establishes its unique oscillatory pattern is unknown and a nodal regulator that allows integration of central and peripheral information and coordinates cardiac circadian output gene expression has been elusive. Using RNAseq in a circadian fashion, we uncovered a biphasic transcriptomic oscillation in the murine heart with a distinct maximum energy production phase and a remodeling and repair phase corresponding to the active and resting phase of a rodent. This biphasic landscape of gene expression requires a transcription factor, Kruppel-like-factor 15 (KLF15). Depletion of KLF15 specifically in the cardiomyocytes leads to a disorganized oscillatory gene expression despite an intact core clock. We show that KLF15 plays a dominant role in the active phase in regulating energy production by directly promotes expression of multiple genes in the fatty acid and amino acids catabolic pathways, which are essential for energy production from food sources. This result is further supported by our previous work demonstrating loss of KLF15 disrupts energy metabolism in the heart and leads to exaggerated eccentric cardiomyopathy. Further, we show that KLF15 is required to recruit circadian repressor REV-ERB$_{\alpha}$ and NCOR to maintain quiescent expression of a subset of genes, which may oscillates in a different tissue context. And depletion of KLF15 results in futile and potentially harmful oscillation in gene expression in response to fluctuating cellular environment. Thus, we identified KLF15 as the crucial nodal connection between the clock and meaningful rhythmicity in the heart. KLF15 deficiency is observed in human hearts with both ischemic and non-ischemic heart failure. Our results open the window for chronotherapeutic options for the prevention and treatment of heart failure.

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Alkylphosphocholine Analogs for Broad Spectrum Cancer Imaging and Therapy

***Ray Zhang*[1], Jamey Weichert[2], Tej Mehta[3], Paul Clark[4], Anatoly Pinchuk[5], Marc Longino[6], Abram Vaccaro[7], William Clarke[8], Mohammed Farhoud[9], Kyle Swanson[10], John Kuo[11]**

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CLR1404 was selected among APC analogs as the best tumor-imaging agent, showing tumor-selective uptake and retention in over 55 different spontaneous and transgenic models of rodent and human cancers. CLR1404 analogs enter via lipid rafts, which are more abundant in cancer cells relative to normal cells, and display prolonged tumor-selective retention in patient-derived GBM cancer stem cells and xenografts, and low retention in normal stem cells and tissues. Depending on the iodine isotope added, radioiodinated CLR1404 is used as either a PET imaging (^{124}I) or molecular radiotherapeutic (^{131}I) agent. The fluorescent analogs CLR1501 (green fluorescence) and CLR1502 (near infrared) were created for real-time tumor cell visualization. Clinical trials in advanced cancer patients using human PET/CT and SPECT/CT imaging with ^{124}I- or ^{131}I-CLR1404, respectively, demonstrated selective uptake and prolonged retention in both primary and metastatic malignant tumors. To understand how the structure of CLR1404 analogs may influence the distribution and clearance kinetics of these agents, which inform pharmacological safety and dosing, we performed pharmacokinetic studies with CLR1404 analogs. Plasma partitioning studies suggests binding of CLR1404 analogs predominantly to albumin. Crystal structure information on structurally similar molecules, and *in silico* modeling using CLR1404 analogs suggest high-affinity binding to seven distinct sites on human serum albumin. We demonstrate through binding assays, and pharmacokinetic studies using rodents that high-affinity binding to albumin along with physicochemical properties predict distribution and clearance kinetics for CLR1404 analogs more effectively than either alone. CLR1404 represents a new class of synthetic APC analogs useful as broad spectrum, tumor-selective molecular imaging and therapy agents in human cancers. Combined application of these chemically identical APC-based radioisosteres, and an understanding of the distribution and clearance kinetics, will enable personalized dual-modality cancer therapy by using ^{124}I-CLR1404 and fluorescent APC for tumor imaging, and for planning follow-up ^{131}I-CLR1404 therapy.

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Targeting Egr1 Selectively Radioprotects Normal Tissues While Killing Cancer Cells

***Diana Yi Zhao*[1], Keith Jacobs[2], Dennis Hallahan[2], Dinesh Thotala[2]**

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Radiotherapy is a common therapeutic modality for a wide range of malignancies. Normal tissue toxicity reduces the therapeutic index of radiotherapy and decreases the quality of life for cancer survivors. Apoptosis is a key element of the radiation response in normal tissues like the hippocampus and small intestine, and results in neurocognitive disorders and intestinal malabsorption. The Early Growth Response 1 (Egr1) transcription factor mediates radiation-induced apoptosis since it activates the transcription of pro-apoptosis genes in response to ionizing radiation (IR). Therefore, we hypothesized that the genetic abrogation of Egr1 and the pharmacological inhibition of its transcriptional activity could attenuate radiation-induced apoptosis in normal tissues. We demonstrated that *Egr1* null mice had less apoptosis in the hippocampus and intestine following irradiation as compared to their wild-type littermates. Alternatively, mice treated with Mithramycin A, a drug that prevents binding of Egr1 to target promoters, also displayed reduced apoptosis following irradiation in the intestine as compared to mice treated with vehicle. In cell culture based studies, shRNA-mediated silencing of Egr1 expression dampened apoptosis and enhanced the clonogenic survival of irradiated HT22 hippocampal neuronal cells and IEC6 intestinal epithelial cells. Mechanistically, these events involved an abrogation of p53 induction by IR and an increase in the ratio of Bcl-2/Bax expression. In contrast, targeted silencing of Egr1 in two cancer cell lines (GL261 glioma cells, HCT116 colorectal cancer cells) was not radioprotective, since it reduced their growth while also sensitizing them to radiation-induced death. These results strongly support the potential of targeted silencing of Egr1 in order to improve the therapeutic index of radiotherapy and minimize the normal tissue complications associated with radiation therapy.

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Ubiquitin E3-Ligase HECTD2 Controls SUMO E3-ligase PIAS1 Stability to Regulate Inflammation

***Joe Zhao*, Bill Chen**

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PIAS1 (protein inhibitor of activated STAT1) is a multi-functional and potent anti-inflammatory protein that negatively regulates several key inflammatory pathways such as JAK-STAT and NF-kB. Discovered here is a novel ubiquitin E3 ligase, HECTD2, that ubiquitinates and mediates the degradation of PIAS1, thus increasing inflammatory responses. Importantly, glycogen synthase kinase (GSK3β) binds and phosphorylates PIAS1 serving as a phosphodegron motif for HECTD2 target initiation. We identified a naturally occurring HECTD2 polymorphism (HECTD2^{A19P}) in 8.5% of the population that functions to blunt inflammatory responses by exclusively causing the mislocalization of HECTD2^{A19P} in the cytosol preventing nuclear interaction with

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PIAS1. We also developed a small molecule inhibitor, BC-1382, to target HECTD2 and attenuates pro-inflammatory stimuli-induced activation of NF-κB. These studies provide a new innate immunity pathway suggesting that mutation or antagonism of E3 ligase HECTD2 results in reduced severity of inflammation by selectively modulating the abundance of anti-inflammatory protein PIAS1.

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Optimally Combining CD3z, CD28 and 4-1BB Signals in 1928z-41BBL CAR T Cells Results in Balanced T Cell Effector Function and Proliferation

Zeguo Zhao, **Sjoukje van der Stegen, Maud Condomines, Fabiana Perna, Gertrude Gunset, Jason Plotkin, Michel Sadelain**

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Chimeric antigen receptors (CARs) are a novel class of drugs consisting in synthetic receptors that redirect and reprogram T cell function. We have demonstrated, in xenogeneic tumor models and later in leukemia patients, that CAR-modified, CD19-targeted T cells can induce complete remissions of relapsed or refractory B cell malignancies. The costimulatory signals provided by second generation CARs and endogenous costimulatory receptors are critical in shaping the quality, potency and durability of T cell responses. Here we demonstrate that optimally combined CD3z, CD28 and 4-1BB signals not only augment T cell proliferation and effector function, but also activate the endogenous interferon-ß (IFNß) pathway in an IRF-7-dependent manner, enabling very small T cell doses to eradicate systemic established leukemia. This unexpected role of the IRF-7/IFNb pathway provides new insights into costimulatory synergy and a novel mechanism for engineered T cells through which to enhance tumor eradication.

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The Role of Sirt2 in Regulating the Critical Basal-like Breast Cancer Determinant Slug

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Breast cancer is a strikingly heterogeneous disease comprised of a multitude of discrete molecular subtypes. Basal-like breast cancer (BLBC) represents the deadliest form of these subtypes, and affects 20% of breast cancer patients. Unlike other subtypes, few driver mutations have been identified for BLBC and, thus, no targeted therapies have been established. Moreover, knowledge of the molecular processes underlying BLBC is limited. We previously reported that the transcriptional repressor Slug is frequently overexpressed in BLBC patients harboring *BRCA1* mutations, and may in fact represent an important determinant in BLBC genesis and progression. Notably, Slug is a short-lived protein that is strictly regulated by proteasomal degradation in normal breast tissue. The mechanism by which it becomes deregulated in cancer remains unknown. Thus we performed a chemical inhibitor screen to identify potential homeostatic regulators of Slug. Strikingly, we found that Sirt2 inhibition leads to rapid turnover and depletion of Slug protein, whereas Sirt2 overexpression stabilizes Slug and increases its level and activity. Mechanistically, we showed that Sirt2 binds to and deacetylates Slug protein to protect it from ubiquitination-mediated proteasomal degradation, thereby stabilizing Slug protein and increasing its bioavailability. Analysis of nearly 1,000 patients from *The Cancer Genome Atlas* (TCGA) dataset revealed that basal-like breast tumors more frequently exhibited Sirt2 copy number amplification compared to other breast cancer subtypes (23% in BLBC, $P<0.05$). Furthermore, we found that Sirt2 knock-down in BLBC cell lines abolishes Slug stability, leading to decreased Slug abundance and the suppression of metastasis-associated markers of the epithelial-to-mesenchymal transition (EMT). Taken together, these results illuminate a regulatory mechanism by which the transcriptional repressor Slug can be dynamically regulated by Sirt2-dependent deacetylation. Co-opting this molecular mechanism to aberrantly stabilize transcriptional repressors such as Slug could be a general feature of cancer cells for adopting malignant behaviors. As such, we are currently examining whether targeting Sirt2 could be a rational strategy to molecularly dampen Slug hyperactivity and thereby alleviate BLBC development and progression.

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High Fat Diet Promotes Tumorigenesis in p53-Null Mammary Epithelium

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p53 mutations are found in 27–58% of all human breast cancer, and are highly associated with triple-negative tumors and poor prognosis. From epidemiological and animal studies, high fat diet (HFD) has been implicated as a potential risk factor for breast cancer. We investigated the effects of HFD on mammary tumorigenesis in wild-type BALB/c mice receiving p53-null mammary transplants. Exposure to continuous HFD from puberty onwards or late exposure to HFD in adulthood increased the incidence of mammary tumors. Notably, late HFD specifically increased the incidence of spindle cell carcinomas that resemble claudin-low triple negative breast cancer. Irrespective of histopathology, tumors developed in mice fed continuous HFD, early HFD limited to puberty and late HFD limited to adulthood all showed enhanced tumor proliferation, angiogenesis and macrophage recruitment. HFD promotes mammary tumorigenesis in the p53-null tumor model and uniquely leads to the development of a subset of tumors that resemble a type of triple negative breast cancer (TNBC) in humans, indicating that this may be a useful animal model for TNBC.

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Altered Processing of the Mitochondrial Genome may Link Known Molecular and Synaptic Abnormalities in Autism Spectrum Disorder

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Both gene expression and mitochondrial function are known to be abnormal in the brains of individuals with autism spectrum disorder (ASD). However, no work has specifically investigated the expression of the unique mitochondrial genome (mtDNA) in ASD brain. Therefore, I hypothesized that altered expression of the mitochondrial transcriptome may represent a point of convergence between previously reported defects in gene transcription and known synaptic abnormalities related to dysfunctional mitochondrial-initiated apoptosis in ASD. To test this hypothesis, I performed mitochondrially-focused gene expression profiling on post-mortem brain tissue from pediatric autistic patients (n =9), and matched control samples (n = 9). The prefrontal cortex (PFC) and the cerebellum, both previously associated with ASD, were assessed from each donor. A human mitochondria-focused cDNA microarray was designed, which contained all 37 mitochondrial–encoded genes, 1,098 nuclear–encoded genes that function specifically in the mitochondria, and 225 controls. Microarray labeling, hybridization, and bioinformatic data analysis were performed following standard protocols. Our results demonstrate substantial, brain-region specific changes in both mtDNA and nuclear-encoded genes with mitochondrial function in ASD. Specifically, all mtDNA genes that are up-regulated in autistic PFC encode for transfer RNAs (tRNAs). In the cerebellum, most of the same tRNAs are also up-regulated, but so are components of the NADH dehydrogenase complex. Remarkably, nuclear-encoded mitochondrial genes with significantly increased expression in ASD prefrontal cortex (274 genes) are enriched for the process of apoptosis, whereas significantly down-regulated genes in autistic PFC (197 genes) are enriched for the process of oxidative metabolism. However, autistic cerebellum does not have apoptosis-related gene expression changes. In summary, autistic brains have alterations of mitochondrial genes involved in oxidative metabolism and in tRNAs in both PFC and cerebellum, yet apoptotic functions appear up-regulated only in ASD prefrontal cortex. This supports other cell-level work in ASD of PFC-specific mitochondrial abnormalities. Collectively, our results suggest that altered processing of the unique mitochondrial genome may partially reconcile known transcriptional and mitochondrial/apoptosis abnormalities in ASD, and these molecular results recapitulate known cell-level region-specific pathology.

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Role of the Coagulation Factor XI in Promoting Distal Platelet Aggregation and Thrombus Formation in Whole Blood Under Flow

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Background: Exposure of the extracellular matrix proteins, such as collagen, and tissue factor (TF), at sites of vascular or tissue injury, serve to initiate platelet recruitment and activation as well as thrombin generation and fibrin formation commencing in a hemostatic plug. Coagulation factor XI (FXI) is a serine protease shown to play a role in the amplification of thrombin generation and inhibition of fibrinolysis via a feedback loop in plasma. FXI activity has been shown to contribute to thrombotic complications, such as disseminated intravascular coagulation, which is characterized by the occlusion of small vessels and widespread consumption of platelets. However, the mechanism by which FXI promotes platelet activation in blood, under physiologically-relevant shear flow conditions, is ill-defined. **Aims:** To determine the role of FXI in promoting local and distal thrombin generation, platelet activation, and occlusive thrombus formation. **Methods:** Whole blood was perfused over a surface of immobilized collagen alone or in the presence of TF at either venous or arterial shear rates. Local platelet aggregation and fibrin formation was quantified by light microscopy and Western blot. Distal blood samples were collected downstream at equivalent mean residence times; reactions were quenched with the serine protease inhibitor PPACK, and were analyzed by FACS. In select experiments, blood was pretreated with the anti-FXI mAb, 1A6, which inhibits FXI activation by FXIIa and FIX activation by FXIa. **Results:** Perfusion of whole blood over collagen resulted in local platelet adhesion, aggregation and fibrin formation. Inhibition of FXI activity abrogated local fibrin formation on collagen without affecting the extent of platelet adhesion and aggregation. Robust platelet aggregation and eventual consumption was observed as a function of time in downstream samples. Inhibition of FXI activity dramatically reduced the rate and extent of downstream platelet aggregation. Our results show that the effect of FXI in promoting local fibrin formation and distal platelet aggregation was dependent upon the extent and trigger of local thrombin generation and physical parameters of residence time and shear rate. **Summary/Conclusions:** Disseminated intravascular coagulation (DIC) is characterized by consumption of platelets and coagulation factors, resulting in both occlusive thrombosis and secondary hemorrhage. Current anticoagulation strategies to restore organ perfusion in DIC are limited due to their inherent risk of increased bleeding. Our results demonstrate that targeted inhibition of FXI reduces distal platelet activation and occlusive thrombus formation without affecting local hemostasis. This study may provide a rationale for the development of FXI inhibitors for the safe and efficacious treatment of thrombotic complications without increased bleeding.

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Loss of Endothelial Surface Glycocalyx (ESG) in Early Sepsis

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Background: ESG represents a surface layer consisting of glycoproteins, proteoglycans and glycosaminoglycans, of which heparan sulfate (HS) is the most abundant. ESG provides a barrier to water and solute transport, interaction between circulating and endothelial cells, sensing of mechanical forces, and shielding receptors from hyper-activation. Sepsis disorganizes ESG, but the mechanism is nebulous. Among the earliest responses of activated endothelial cells to LPS are exocytosis of Weibel-Palade bodies (WPB) and lysosomes. We hypothesized that exocytosis of WPB and lysosomes, both very early responses of endothelial cells to endotoxin, results in the focal degradation of endothelial glycocalyx, unleashing the cascade of events culminating in multiorgan failure. **Methods:** bEnd3 endothelial cells and HUVEC were treated with LPS (5ug/ml) and stained with mAb against HS or vWF; intravital-microscopy of lysosomal motility was performed using LysoTracker followed by point-tracking. In vivo, C57BL/6 mice were injected with LPS IP. After 1 h or 6h treatment, the cremaster microvessels were in vivo stained with FITC-anti-HS; in another group, the aorta was similarly stained en face. Zeiss LSM 710 confocal microscopy and 3-D multi-color ultra-high resolution Nikon-STORM (Stochastic Optical Reconstruction Microscopy) were used to detect ESG, vWF/WPB and lysosomes. **Results:** At 10 min after LPS treatment of bEnd3, we observed a significant loss of ESG and exocytosis of vWF/WPB (Fig. 1). At 30 min, ESG was degraded by -90%, 30% more lysosomes and 4-fold vWF /WPB externalized compared to the control. 1 h and 6h LPS treatment removed ESG by -50% and more than -90%, respectively, in microvessels; by -60% and -90%, respectively, in aorta of mice. **Conclusions:** Data shows that after 10-30 min of LPS patchy loss of ESG is detectable coinciding with exocytosis of WPB and lysosomes in cultured cells. Experiments confirmed that the loss of ESG is an early event in sepsis. Our findings suggest an effective therapeutic target in preventing sepsis complications by preserving ESG.

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Spatio-temporal Controlled Release of Dopamine as a Novel Treatment for Parkinson's Disease

***Muna Oli*[1], Takashi Nakano[2], Jeff Wickens[2]**
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Parkinson's Disease (PD) affects over one million Americans currently, with 60,000 being diagnosed every year. Biologically, PD causes the degeneration of dopaminergic nigro-striatal neurons in the basal ganglia, leading to decreased dopamine secretion. Currently the most common medication to treat PD is Levodopa which has many side effects such as the "wearing off effect" and the "on-off phenomenon," making it a short term treatment option for PD patients. It is evident that there is an acute need to develop a highly specific, targeted and remote controlled release of dopamine that simulates dopamine release by neurons to treat PD on demand and long term without the side effects and reduced efficacy. Through the incorporation of liposomes, nanoparticles, dopamine and a femtosecond near infrared laser, we hope to construct a drug delivery system that can be remotely controlled with high precision and accuracy, and allows drug release of dopamine from the liposomes in small amounts at a time, simulating a neuron secreting dopamine. The incorporation of liposomes increases the bioavailability and half-life of dopamine, and the use of a near infrared laser reduces the potential for any damage to surrounding tissues. This research focuses on: 1) synthesis, optimization and characterization of gold nanoparticles for the drug delivery system, 2) testing the efficacy of the drug delivery system with the different types of gold nanoparticles, and 3) designing a better way to determine drug release.


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Notes

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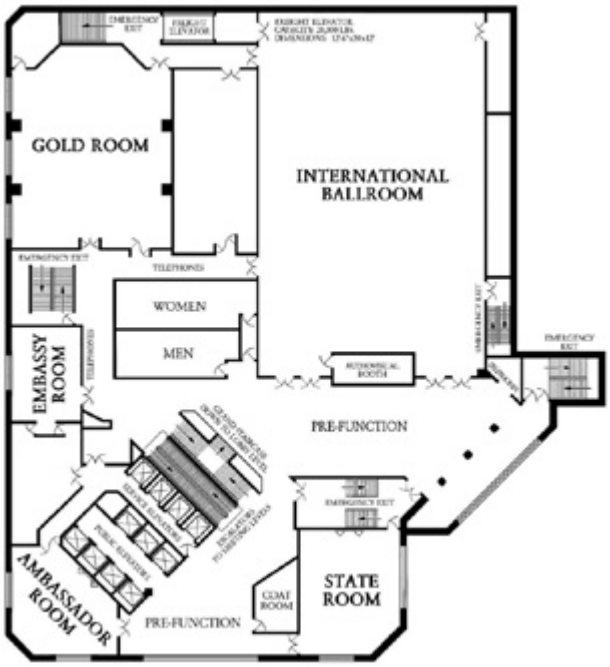
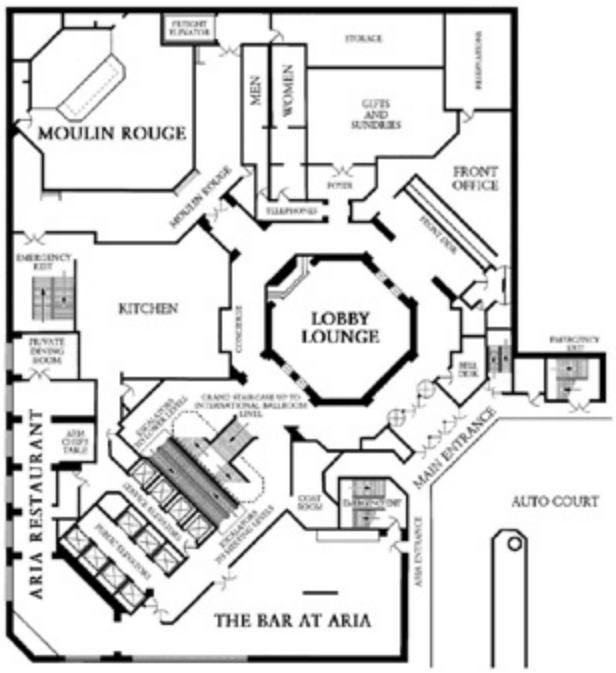
Hotel Floor Plans

International Ballroom Level (2nd level)

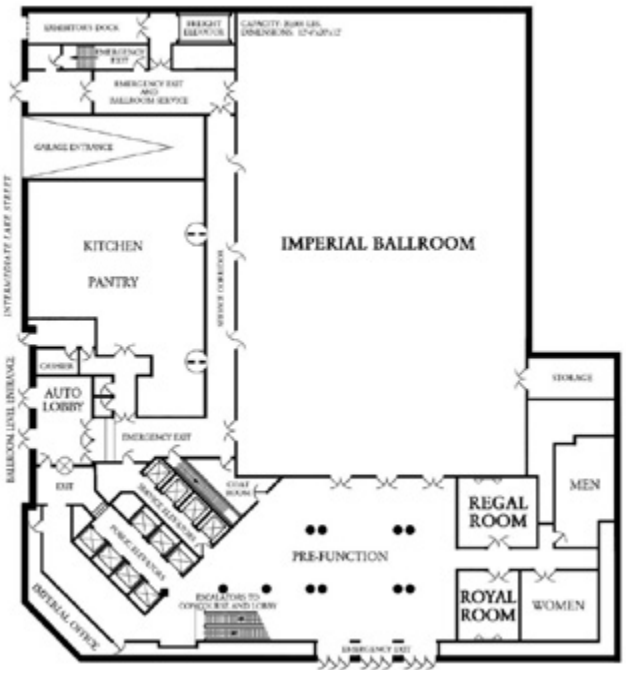


Meeting Room Level (3rd level)

Lobby Level (1st level)



Imperial Ballroom Level (B2 level)



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