AAP • ASCI • APSA Joint Meeting

1886 Celebrating the 130th Meeting of the Association of American Physicians 2016

April 15 – 17, 2016

Fairmont Chicago Millennium Park • Chicago, Illinois

BOSTON UNIVERSITY

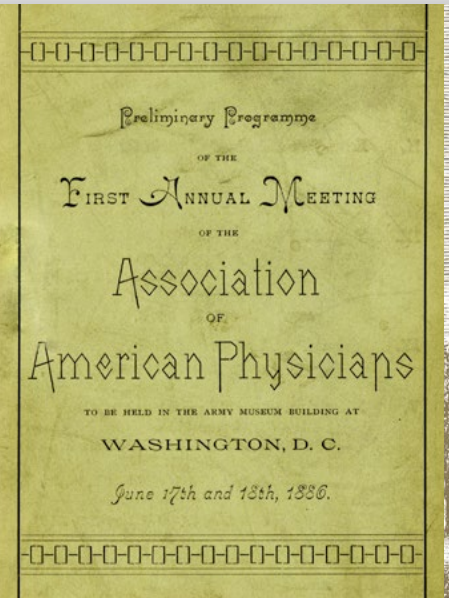
Boston University School of Medicine
Continuing Medical Education

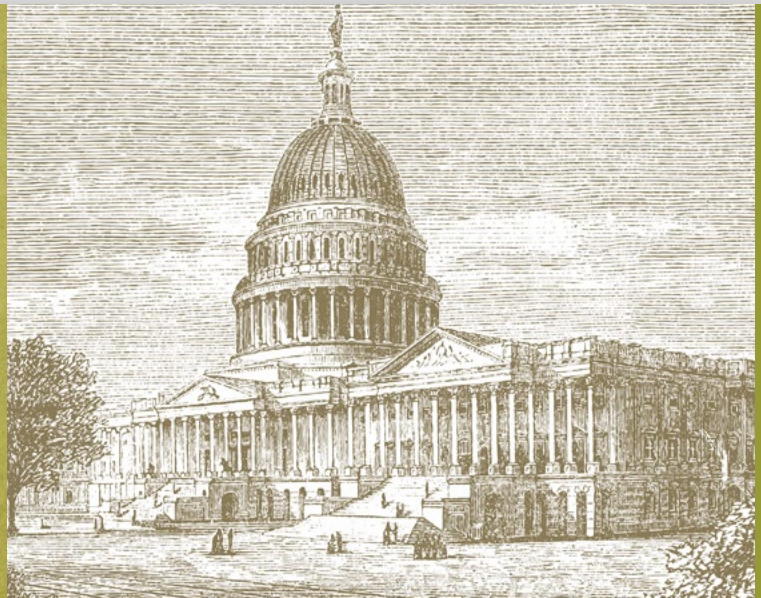
The AAP/ASCI/APSA conference is jointly provided by Boston University School of Medicine and AAP/ASCI/APSA.

Meeting Program and Abstracts

APSA
American Physician Scientists Association

www.jointmeeting.org


Preliminary Programme
OF THE
First Annual Meeting
OF THE
Association
OF
American Physicians
TO BE HELD IN THE ARMY MUSEUM BUILDING AT
WASHINGTON, D. C.
June 17th and 18th, 1886.



130 Anniversary 1886-2016

Special Events at the 2016 AAP/ASCI/APSA Joint Meeting

Friday, April 15

ASCI President's Reception

6:15 – 7:15 p.m.
Gold Room

ASCI Dinner & New Member Induction Ceremony

(Ticketed guests only)

7:30 – 9:45 p.m.
Rouge, Lobby Level

Speaker: **Clara D. Bloomfield, MD**
The Ohio State University Comprehensive Cancer Center

APSA Welcome Reception & Presidential Address

9:00 p.m. – Midnight
Signature Room, 360 Chicago,
John Hancock Center (off-site)

Speaker: **Daniel DelloStritto, APSA President**

www.jointmeeting.org

Saturday, April 16

ASCI Food and Science Evening

6:30 – 9:00 p.m.
The Mid-America Club, Aon Center

AAP Member Banquet

(Ticketed guests only)

7:00 – 10:00 p.m.
Imperial Ballroom, Level B2

How to Solve a Scientific Puzzle:
Clues from Stockholm and Broadway

Speaker: **Joe Goldstein, MD**
University of Texas Southwestern Medical Center at Dallas

APSA Dinner

(Ticketed guests only)

7:30 – 9:00 p.m.
Rouge, Lobby Level

Finding One's Scientific Niche:
Musings from a Clinical Neuroscientist

Speaker: **Helen Mayberg, MD,** *Emory University*

Dessert Reception

(open to all attendees)

10:00 p.m. – Midnight
Imperial Foyer, Level B2

Sunday, April 17

APSA Future of Medicine and Residency Luncheon

Noon – 2:00 p.m.
Rouge, Lobby Level

Program Contents

General Information

Registration Desk Hours

Friday, April 15	7:00 a.m. – 6:30 p.m.
Saturday, April 16	7:00 a.m. – 5:00 p.m.
Sunday, April 17	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.

Joint Meeting Evaluations

The AAP/ASCI/APSA Joint Meeting Planning Committee relies on your input to enhance its meetings. Following the Joint Meeting, an online meeting evaluation will be emailed to all attendees. APSA attendees will receive a separate survey to help its planning committee enhance APSA-sponsored events at future AAP/ASCI/APSA Joint Meetings. Your participation in this survey is greatly appreciated.

AAP/ASCI/APSA Joint Meeting Code of Conduct

We value your attendance. Our conference is dedicated to providing a harassment-free experience for everyone, regardless of gender, gender identity and expression, age, sexual orientation, disability, physical appearance, body size, race, ethnicity, or religious preference. AAP/ASCI/APSA do not tolerate harassment of conference participants in any form. A participant engaging in harassing behavior will be warned and may be asked to leave the conference with no refund. If you are being harassed, notice that someone else is being harassed, or have any other concerns, please contact a member of conference staff at the registration desk immediately. Conference staff and organizers are dedicated to making all participants feel safe for the duration of the conference.

Poster Session Schedule

Friday, April 15

1:00 p.m. – 3:00 p.m.	Poster Setup
6:15 p.m.– 9:30 p.m.	Informal Viewing: presenters do not need to be at poster

Saturday, April 16
TWO Poster Presentation Sessions

8:00 a.m. – 9:30 a.m.	Poster Session with Continental Breakfast **ODD numbered posters presented** Featuring presentations by the ASCI's 2016 Young Physician-Scientist Awardees
11:45 a.m. – 1:30 p.m.	Poster Session with Lunch **EVEN numbered posters presented**
1:30 p.m. – 2:00 p.m.	Poster Dismantle
2:45 p.m. – 3:00 p.m.	Outstanding Poster Awards (during Plenary III in International Ballroom)

Poster abstract submitters should plan to be available on Saturday for their appointed poster presentation session and the resulting awards program later in the afternoon.

Outstanding Poster Awards

Best Poster Awards will be given in the amount of $1,000 each. Members of the AAP, ASCI and APSA will judge posters on scientific novelty, quality and clarity of presentation. Awards will be presented on Saturday, April 16, from 2:45 – 3:00 p.m.

Continuing Medical Education Information

Overview

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 2016 AAP/ASCI/APSA 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.

Claiming CME Credit

Participants will receive a CME Personal Attendance Log with conference materials. The log includes instructions for completing the online CME application and evaluation. In order to receive credits this online evaluation must be completed. CME Certificates will be sent within 8 weeks of completing the application. Feel free to contact LaTanya Morris at lmorris@jointmeeting.org with any questions.

Accreditation and Credit Designation

This activity has been planned and implemented in accordance with the accreditation requirements and policies of the Accreditation Council for Continuing Medical Education through the joint providership of Boston University School of Medicine, the American Society for Clinical Investigation, the Association of American Physicians and the American Physician Scientists Association.

Boston University School of Medicine designates this live activity for a maximum of 8.5 *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

Christine E. Seidman, MD
AAP President
Brigham and Women's Hospital

Linda Fried, MD
AAP Vice President
Columbia University, Mailman School of Public Health

Paul Rothman, MD
AAP Immediate Past President
Johns Hopkins University

Dan DelloStritto
(6th year MD/PhD)
Northeast Ohio Medical University

Jillian Liu
(4th Year MD/PhD)
The Ohio State University

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

Vivian Cheung, MD
ASCI President-Elect
Howard Hughes Medical Institute, University of Michigan

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

Lori Ennis
Executive Director AAP, Ex Officio Committee Member

John Hawley
Executive Director ASCI, Ex Officio Committee Member

Continuing Medical Education Information

Learning Objectives

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

- Describe important recent advances in the scientific basis of disease and therapy
- Describe novel strategies to address challenges to the physician-scientist
- Describe 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 2016 AAP/ASCI/APSA Joint Meeting program is organized into several plenary sessions that have broad applicability: "Good and Bad Bugs" (focused on infectious disease and immunology), "Epigenetics and Transcriptional Regulation of Human Disease" (external influences on gene switching), "Cool Tools and Forward Technology" (tissue engineering and drug discovery), and "Metabolism" (regulation of the endocrine system and drug development).

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.

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.

Disclosure Policy

The University of Boston School of Medicine requires faculty and members of the planning committee to disclose whether or not they have any relevant commercial relationships or if they will be discussing unlabeled and/or investigational uses of any products, pharmaceuticals, or medical devices. This must be made known in advance to the audience in accordance with the ACCME Standards of Commercial Support guidelines.

Program Committee Disclosures

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

First	Last	Financial Disclosure
Levi	Garraway	Research Support, Novartis Consultant, Foundation Medicine, Novartis, Boehringer Ingelheim. Equity holder, Foundation Medicine. Scientific Advisory Board, Warp Drive
Paul	Rothman	Board of Directors, Merck
Christine	Seidman	Founder and Shareholder, Myokardia Inc.

Abstract Reviewer Disclosures

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

John	Belmont	Employed as Sr. Principal Medical Scientist, Illumina, Inc.
Jeffrey	Borer	Consultant, Servier, Cardiorentis, ARMGO, Novartis, Celladon, Amgen- Speakers Bureau, Pfizer, Takeda USA, Bayer, and AstraZeneca. Minor Stockholder, BioMARIN.
Renier	Brentjens	Co-founder, Stockholder and Consultant, Juno Therapeutics Inc.
Sam	Dudley	Major stockholder, 3PrimeDX.
Andrew	Einstein	Employer (Columbia University) Grant/Research Support, GE Healthcare, Philips Healthcare, Toshiba America Medical Systems.
David	Ellison	Spouse, consultant, HeartWare.
Don	Ganem	Employee, Stockholder, Novartis.
Iris	Jaffe	Grant, Novartis.
Jay	Kolls	Consultant, Boehringer-Ingelheim.
Vivian	Lee	Board of Directors, Merrimack Pharmaceuticals, Zions Bank.
Dermot	McGovern	Consultant, MERCK, Cidara, Jansen and Jansen, UCB. Research Support, Amgen.
Shlomo	Melmed	Consultant, Chiasma, ISIS, Novartis. Research support, Ipsen, Pfizer
Charles	Mullighan	Speaker, Amgen. Consultant, Incyte. Research Funding, Loxo Oncology
Mary-Elizabeth	Patti	Grant Support, Astra-Zeneca - investigator initiated, Medimmune –research, Nuclea/MetaboDx – research, Xeris – collaborative from NIH, Janssen - investigator-initiated.
Jeremy	Rich	Honoraria, Vertex and PTC Therapeutics.

Full Disclosure Policy Affecting CME Activities

Abstract Reviewer Disclosures (continued)

Joel	Schuman	Patents/Royalty: Zeiss. Educational Grant Support: Glaukos, Aerie. Consultant/Advisor: Aerie, Pfizer, Alcon Laboratories, Slack/Vindico. Equity Owner: Ocugenix. Employee, Spouse: Shire – Marketing.
Ramesh	Shivdasani	Research Grant and Consultant, Novartis Oncology.
Kalyanam	Shivkumar	EP Dynamics
Jeffrey	Toretsky	Founders stock and Consultant, EP Dynamics
Kenneth	Tyler	Grants, NIH and Dept. Veterans Affairs and National MS Society (no Pharma grants). Scientific Advisory Boards/Data Safety Monitoring Boards, Genentech, PML Consortium, LPath inc. and in past Pfizer, Roche, Biogen, Janssen (J& J).

2016 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 faculty in the accredited program have completed financial disclosure forms and had no financial relationships to report.

First	Last	Financial Disclosure
Michael	Brown	Director, Regeneron Pharmaceuticals, Inc. and Peloton.
Daniel	Drucker	Advisor/Consultant, Arisaph Pharmaceuticals Inc., Intarcia Therapeutics, Merck Research Laboratories, MedImmune, Novo Nordisk Inc.,., Receptos, and Sanofi, Inc. Partner with University of Toronto to a license agreement with Shire Pharmaceuticals for development of GLP-2-based therapies
David	Tuveson	Grant support: Fibrogen, Halozyme. Consultant: Infinity, Eli Lilly, Warp Drive, Merck Serano, Gilead.
David	Wright	iDETECT - Patent Progesterone for TBI - Patent Astrocyte Pharma - Scientific Advisory Board Sinapsis - Scientific Advisor
Richard	Young	Director and stockholder, Syros Pharmaceuticals
Huda	Zoghbi	Research Support, UCB pharmaceuticals.

Unlabeled/Investigation Uses of Products or Devices

The following faculty indicated that they plan to discuss unlabeled or investigational uses of products or devices. They have agreed to disclose this to participants. All other faculty in the accredited program have completed content validation forms and indicated they would not be discussing unlabeled or investigational uses of any products or devices.

First	Last	Unlabeled or Investigational Use to be Discussed
David	Tuveson	Preclinical investigational therapies and off label experiments will be discussed.

Scientific Program Schedule

Thursday, April 14

Time	Event	Location
5:00 p.m. – 6:30 p.m.	APSA Grant-Writing Workshop Facilitator: **Kathrine Knight, PhD,** *Loyola University Chicago*	Crystal

Friday, April 15

7:00 a.m. – 6:30 p.m.	Registration	International Ballroom Foyer
8:30 a.m. – 11:00 a.m.	APSA Business Meeting	Rouge
11:00 a.m. – 1:00 p.m.	**APSA Session I**	International Ballroom
11:00 a.m. – Noon	 Speaker: **Anna Penn, MD, PhD,** *Children's National Medical Center* *Sponsored by the Society for Pediatric Research and the American Pediatric Society* **Perinatal Brain Damage: A Placental Disorder***	
Noon – 1:00 p.m.	 Speaker: **Diane Griffin, MD, PhD,** *Johns Hopkins University* *Sponsored by the Infectious Diseases Society of America* **Acute Virus Infections: Determinants of Outcome***	
1:00 p.m. – 3:00 p.m.	Poster Setup	Imperial Ballroom
1:00 p.m. – 6:00 p.m.	**Plenary Session I: Good and Bad Bugs** Moderators: **Vivian Cheung, Dan DelloStritto and Linda Fried**	International Ballroom
1:00 p.m. – 1:30 p.m.	 Speaker: **Anthony S. Fauci, MD,** *National Institutes of Health* **Ending the HIV/AIDS Pandemic: An Achievable Goal***	
1:30 p.m. – 2:00 p.m.	 Speaker: **Julie A. Segre, PhD,** *National Institutes of Health* **Skin Microbiome: What's the 'skinny' on Microbiome?** **Interplay of Skin Microbes, Immune Cells, and Barrier in Health and Disease***	
2:00 p.m. – 3:00 p.m.	**ASCI and AAP Invited New Member Presentations***	
2:00 p.m. – 2:15 p.m.	 ASCI New Member: **Andy Minn, MD** *University of Pennsylvania* **Response and Resistance to Immune Checkpoint Blockade in Mice and Patients**	
2:15 p.m. – 2:30 p.m.	 AAP New Member: **Edwin M. Stone, MD, PhD** *University of Iowa Carver College of Medicine* **Curing Inherited Blindness**	
2:30 p.m. – 2:45 p.m.	 ASCI New Member: **Sallie Robey Permar, MD, PhD** *Duke University* **Protecting the Next Generation: Maternal Immunization to Prevent Perinatal Infections**	
2:45 p.m. – 3:00 p.m.	 AAP New Member: **Robert F. Siliciano, MD, PhD** *Johns Hopkins University* **Barriers to Curing HIV Infection**	

**Eligible for CME credit.*

Scientific Program Schedule

Friday, April 15 (continued)

Time	Event	Location
2:00 p.m. – 3:00 p.m.	**APSA Panel Discussion: Key Issues Facing Physician–Scientists** *Sponsored by Novartis Institutes for BioMedical Research Inc.* Moderator: **Jennifer Kwan, MD, PhD,** *University of Illinois at Chicago, APSA Policy Chair* Panelists:  **Victor Dzau, MD** **President** *National Academy of Medicine* *(formerly the Institute of Medicine)*  **Stephen Ostroff, MD** *Former Acting Commissioner,* *Food and Drug Administration*  **Craig Basson, MD, PhD** *Vice President and Global Head,* *Translational Medicine* *(Cardiovascular and Metabolism), Novartis*  **Jennifer Zeitzer, PhD** *Director, Legislative Relations, FASEB*	Gold Room
3:00 p.m. – 3:30 p.m.	Refreshment Break	International Ballroom Foyer
3:30 p.m. – 6:00 p.m.	Moderators: **Christine Seidman, Levi Garraway, Jillian Liu**	International Ballroom
3:30 p.m. – 4:00 p.m.	 **ASCI / Harrington Prize Lecture** **Jeffrey M. Friedman, MD, PhD** *The Rockefeller University*	
4:00 p.m. – 4:30 p.m.	 **APSA Keynote: Gary Nabel, MD, PhD** *Sanofi* **Today's Immunology:** **Leading Tomorrow's Fight Against New Viruses and Cancer**	
4:30 p.m. – 5:00 p.m.	 **ASCI Presidential Address** **Levi Garraway, MD, PhD** *Harvard University* **Believe the Miracles: Of Biomedical Science and Human Suffering**	
5:00 p.m. – 5:30 p.m.	 **ASCI – Stanley J. Korsmeyer Award Lecture** **Jean-Laurent Casanova, MD, PhD** *The Rockefeller University*	
5:30 p.m. – 6:00 p.m.	 **APSA Lasker Foundation Award Lecture** *Sponsored by the Albert and Mary Lasker Foundation* **Michael Brown, MD** *University of Texas Southwestern Medical Center* **Why Prizes?***	
6:15 p.m. – 7:15 p.m.	**ASCI President's Reception**	Gold Room
6:15 p.m. – 9:30 p.m.	Poster Viewing (no presentations)	Imperial Ballroom

**Eligible for CME credit.*

Scientific Program Schedule

Friday, April 15 (continued)

Time	Event	Location
7:30 p.m. – 9:45 p.m.	 **ASCI Dinner & New Member Induction Ceremony** Dinner Speaker: **Clara D. Bloomfield, MD** *The Ohio State University Comprehensive Cancer Center* **Advances in Acute Leukemia:** **Believe What You See, Not What They Say**	Rouge
9:00 p.m. – Midnight	 **APSA Welcome Reception &** **APSA Presidential Address** Speaker: **Daniel DelloStritto, President, APSA**	Off–Site Signature Room John Hancock Center

Saturday, April 16

7:00 a.m. – 5:00 p.m.	Registration	International Ballroom Foyer
7:00 a.m. – 8:00 a.m.	AAP Council Meeting (for AAP Council members only)	State Room
7:00 a.m. – 8:00 a.m.	Mentoring Breakfast	Rouge
8:00 a.m. – 9:30 a.m.	**Poster Presentations and Continental Breakfast** ODD number posters will be presented/judged Also featuring presentations by the ASCI's 2016 Young Physician-Scientist Awardees	Imperial Ballroom
9:30 a.m. – 11:45 a.m.	**Plenary Session II: Epigenetics and Transcriptional Regulation of Human Disease** Moderators: **Vivian Cheung, David Ginsburg and Peter Mittwede**	International Ballroom
9:30 a.m. – 10:00 a.m.	 Speaker: **Richard A. Young, PhD** *Whitehead Institute for Biomedical Research* **Transcriptional and Epigenetic Control of Cell Identity***	
10:00 a.m. – 10:30 a.m.	 Speaker: **Stuart Orkin, MD** *Dana-Farber Cancer Institute* **Solving the Human Fetal to Adult Hemoglobin Switch***	
10:30 a.m. – 10:45 a.m.	**APSA Trainee Oral Abstract Presentation** **Kevin Bersell,** *Vanderbilt University* **Genomic editing in iPSCs establishes that a rare TBX5 variant causes Brugada syndrome**	
10:45 a.m. – 11:15 a.m.	 Speaker: **Mike Snyder, PhD** *Stanford University* **Personalized Medicine: Integrative Omics for Managing Health and Disease***	
11:15 a.m. – 11:45 a.m.	 **APSA Keynote Speaker:** **Huda Zoghbi, MD** *Baylor College of Medicine* **Genetic Approaches to Tackle Neurodegenerative Disorders***	
11:45 a.m. – 1:30 p.m.	**Poster Presentations with Lunch** — EVEN number posters will be presented/judged	Imperial Ballroom
12:45 p.m. – 1:30 p.m	Poster Reviewer Meeting	Royal Room
1:30 p.m. – 2:00 p.m.	Poster Dismantle	Imperial Ballroom

**Eligible for CME credit.*

Scientific Program Schedule

Saturday, April 16 (continued)

Time	Event	Location
1:30 p.m. – 2:45 p.m.	**Plenary Session III: Cool Tools and Forward Technology** Moderators: **Alex Adami, Ben Ebert, and Serpil Erzurum**	International Ballroom
1:30 p.m. – 2:00 p.m.	 Speaker**: David Tuveson, MD, PhD** *Cold Spring Harbor Laboratory* **Insights from Studies of Pancreatic Cancer Organoids***	
2:00 p.m. – 2:15 p.m.	**APSA Trainee Oral Abstract Presentation** **Tiffany Fleet,** *Baylor College of Medicine* **SRC-2 Integrates Polygenic Inputs to Maintain Glucose Homeostasis**	
2:15 p.m. – 2:45 p.m.	 Speaker: **Robert S. Langer, PhD** *Massachusetts Institute of Technology* **Drug Delivery and Tissue Engineering**	
2:45 p.m. – 3:00 p.m.	Outstanding Poster Awards	International Ballroom
3:00 p.m. – 3:30 p.m.	Refreshment Break	International Ballroom Foyer
3:30 p.m. – 5:45 p.m.	**Plenary Session IV: Metabolism** Moderators: **Karen Doersch, Ben Ebert, Paul Rothman**	International Ballroom
3:30 p.m. – 4:00 p.m.	 Speaker: **David Altshuler, MD, PhD** *Vertex Pharmaceuticals* **Human Genetics and the Discovery of New Medicines**	
4:00 p.m. – 4:30 p.m.	 Speaker: **Daniel J. Drucker, MD** *University of Toronto* **Enteroendocrine Therapies for Diabetes, Obesity and Gastrointestinal Disorders***	
4:30 p.m. – 5:00 p.m.	 **AAP Presidential Address** **Christine E. Seidman, MD** *Brigham and Women's Hospital* **AAP: 130 Years of Biomedical Research & Much More Still To Do**	
5:00 p.m. – 5:45 p.m.	 **Kober Medal Presentation** Recipient: **Peter Agre, MD** (Diane Griffin accepting on behalf of Peter Agre.) *Johns Hopkins University School of Medicine*  Presenter: **Paul B. Rothman, MD** *Johns Hopkins University School of Medicine*	

**Eligible for CME credit.*

Scientific Program Schedule

Saturday, April 16 (continued)

Time	Event	Location
6:00 p.m. – 7:00 p.m.	**APSA Panel Discussion: Couples in Medicine** Moderator: **Mariam Bonyadi,** *University of Illinois at Urbana–Champaign* Panelists:  **Parisa Lotfi, MD** *Brigham and Women's and Faulkner Hospital*  **Mehra Golshan, MD** *Dana-Farber Cancer Institute*  **Christine E. Seidman, MD** *Harvard University*  **Jonathan G. Seidman, PhD** *Harvard University*	State Room
6:30 p.m. – 9:00 p.m.	**ASCI Food and Science Evening** Honoring New ASCI Members & Young Physician-Scientist Awardees Featuring light dinner, wine and presentations by award-winning chefs, including Top Chef Masters winner Floyd Cardoz and New York City Michelin Star recipient Ben Pollinger, as well as poster presentations by ASCI Young Physician-Scientist Awardees & HHMI Medical Fellows	The Mid-America Club at the Aon Center (connected by pedway to the Fairmont)
7:00 p.m. – 10:00 p.m.	 **AAP Member Banquet** Dinner Speaker: **Joe Goldstein, MD** *University of Texas Southwestern Medical Center at Dallas* **How to Solve a Scientific Puzzle: Clues from Stockholm and Broadway**	Imperial Ballroom, Level B2
7:30 p.m. – 9:00 p.m.	 APSA Dinner (ticketed event) Speaker: **Helen Mayberg, MD,** *Emory University* **Finding One's Scientific Niche:** **Musings from a Clinical Neuroscientist**	Rouge
10:00 p.m. – Midnight	Dessert Reception (open to all attendees)	Imperial Foyer, Level B2

**Eligible for CME credit.*

Scientific Program Schedule

Sunday, April 17

Time	Event	Location
7:30 a.m. – 10:00 a.m.	Registration	International Ballroom Foyer
7:30 a.m. – 9:00 a.m.	**AAP/ASCI/APSA Council Wrap-up Meeting** (Invited guests only)	State Room
8:00 a.m. – Noon	**APSA Program**	
8:00 a.m. – 9:00 a.m.	APSA Interest Group & Mentorship Breakfast	Rouge
9:00 a.m. – 10:00 a.m.	 **APSA Keynote Speaker:** *Sponsored by Society for Academic Emergency Medicine* **David Wright, MD,** *Emory University* **Translating Basic Science into Successful Clinical Trials: Is There a Path for Neuroprotection?***	Rouge
10:00 a.m. – 11:00 a.m.	**APSA Panel Discussion: Post–Graduate Opportunities** Moderator: **Lillian Zhang,** *University of California, Davis* Panelists:  **Robert Satcher, MD, PhD** *Stanford University*  **Christopher Hsu, MD, PhD** *Centers for Disease Control and Prevention*  **Andrew Lee, PhD** *Stanford University and Co–Founder, StartX Med*	Rouge
10:00 a.m. – 11:00 a.m.	 **APSA Panel Discussion:** **What is Translational Research? Insights from an Investigator Developing Clinical Diagnostic Tools for Early Detection of Neurodegenerative Diseases** Speaker: **Eric Goldwaser, 5th year DO/PhD candidate,** *Rowan University*	Cuvee Room, Lobby Level
11:00 a.m. – Noon	**APSA Panel Discussion: Ethics – Paper Submissions and Data Reproducibility** Moderator: **Hanna Erickson,** *University of Illinois at Urbana–Champaign* Panelists:  **John Ioannidis, MD** *Stanford University*  **Sarah Jackson, PhD** *Executive Editor, the Journal of Clinical Investigation (JCI)*  **Larry Schlesinger, MD** *The Ohio State University*	Cuvee Room, Lobby Level

**Eligible for CME credit.*

Scientific Program Schedule

Sunday, April 17 (continued)

Time	Event	Location
11:00 a.m. – Noon	**APSA Panel Discussion: Technological Innovations** Moderator: **Jennifer Kwan, MD, PhD,** *University of Illinois at Chicago* Panelists:  **Rishi Arora, MD** *Northwestern University Feinberg School of Medicine*  **Gregory Dumanian, MD** *Northwestern University Feinberg School of Medicine*  **Carrie Mendoza, MD** *Advocate Illinois Masonic Medical Center*  **Jake Riggle, PhD** *University of Nebraska Medical Center*  **Maryam Saleh, PhD** *MATTER*	Crystal
Noon – 2:00 p.m.	**APSA Future of Medicine & Residency Luncheon**	Rouge
	UPenn Physician Scientist Residency Program – Dr. Peter Klein	
	Vanderbilt Physician–Scientist Training Program – Dr. Christopher Williams	
	Cincinnati Children's Pediatric Residency/Pediatric Scientist Development Program – Dr. Kathryn Wikenheiser–Brokamp	
	National Institutes of Health – Dr. Frederick Ognibene	
	Brigham and Women's Internal Medicine – Drs. Rebecca Baron, Brittany Weber, and Chris Nabel	
	Children's Hospital Los Angeles Pediatrics/George Donnell Society – Dr. Steven Mittelman	
	University of Iowa Physician–Scientist Training Program – Dr. Joel Kline	
	University of Alabama at Birmingham Medicine Scholar's Program – Dr. Andrea Cherrington	
	Massachusetts General Hospital Internal Medicine Residency and Physician–Scientist Pathway – Dr. Jatin Vyas and Dr. Jay Rajagopal	
	Baylor Pediatrician–Scientist Training & Development Program – Dr. Andrea Burns	
	Ohio State University PSTP – Dr. Robert Baiocchi	
	University of Chicago Pathology – Dr. Kammi Henriksen	
	University of California Los Angeles STAR Program - Dr. Tamer Sallam	
	Yale University, Internal Medicine Research Pathway - Dr. John Wysolmerski	

**Eligible for CME credit.*

Speaker Biographies

Peter Agre, MD

Peter Agre, MD, will be awarded the Association of American Physician's George M. Kober Medal, the organization's highest honor, on Saturday, April 16, for his outstanding contributions to medicine or medical science.

Dr. 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.

David Altshuler, MD, PhD

David Altshuler, MD, PhD, is Executive Vice-President, Global Research and Chief Scientific Officer at Vertex Pharmaceuticals. Dr. Altshuler leads Vertex's research efforts aimed at discovering new medicines for the treatment of serious diseases and oversees the company's five research sites in the United States, Canada and Europe.

Dr. Altshuler was previously one of four founding members, Deputy Director and Chief Academic Officer at the Broad Institute of Harvard and MIT, a professor at Harvard and MIT, and a physician at Massachusetts General Hospital. He was a leader of the SNP Consortium, HapMap and 1,000 Genome Projects, and discovered more than 100 gene variants associated with type 2 diabetes and other common diseases.

A member of the Institute of Medicine and the American Academy of Arts and Sciences, Dr. Altshuler was named a Champion of Change by the White House for his leadership in creating and leading the Global Alliance for Genomic and Health.

Rishi Arora, MD

Rishi Arora, MD, is a cardiologist affiliated with Northwestern Memorial Hospital, Chicago. He received his medical degree from Delhi University College of Medical Sciences. His research interests include the mechanisms and pathophysiology of atrial fibrillation and sudden cardiac death. In 2008 he received an Early Career Development Award from the Central Society for Clinical Research, and has received the Jacque Smith Distinguished Physician Award from Northwestern Memorial Hospital multiple times. He is also a physician scientist entrepreneur who recently founded a company to facilitate translating his patented basic science discoveries in animal models of atrial fibrillation to the market.

Craig Basson, MD, PhD

Craig Basson, MD, PhD, is Vice President and Global Head of Translational Medicine (Cardiovascular and Metabolism) for Novartis Institutes for Biomedical Research.

His team is comprised of talented physician-scientists dedicated to developing novel therapeutics for cardiovascular and metabolic (CVM) disorders. The CVM team in translational medicine is responsible for devising new strategies to assess early efficacy of drugs designed to improve survival and quality of life for patients as well as for identifying specific patient populations that will benefit from these drugs.

Dr. Basson graduated from Washington University in St. Louis, was awarded a Marshall Scholarship at the University of Oxford, and then completed his MD and PhD at Yale University School of Medicine. He trained in internal medicine at Johns Hopkins Hospital and subsequently completed his clinical cardiology fellowship at Harvard University and the Brigham and Women's Hospital along with a research fellowship in human genetics. He began an independent laboratory there before moving to Cornell University Medical College where he was recently Gladys and Roland Harriman Professor of Medicine, Director of the Center for Molecular Cardiology, and the Director of Cardiovascular Research. Dr. Basson was highly recognized for deciphering the molecular genetic basis of inherited congenital and adult onset cardiovascular disease and received several honors including election to the American Society of Clinical Investigation and designation as an Established Investigator of the American Heart Association.

Craig joined NIBR in 2010 as Global Head of Cardiovascular Translational Medicine and assumed the leadership of the combined cardiovascular and metabolism team in 2014.

Clara Bloomfield, MD

Dr. Clara D. Bloomfield earned her MD, from the University of Chicago and completed training in internal medicine and medical oncology at the University of Minnesota, where she became a full professor in just seven years. In 1989, she became professor of medicine and chief of the Division of Oncology at the State University of New York at Buffalo, as well as chair of the Division of Medicine at Roswell Park Cancer Institute. In 1997 she joined The Ohio State University Comprehensive Cancer Center (OSUCCC) as Director. She is the only female professor at OSU to be elected to both the National Academy of Medicine and the American Academy of Arts & Sciences.

Currently, Dr. Bloomfield serves as cancer scholar and senior advisor at the OSUCCC. She is also a member of the Leukemia Research Program, a professor of internal medicine, and she holds the William Greenville Pace III Endowed Chair in Cancer Research. Dr. Bloomfield's 40 years of groundbreaking research on adult leukemia and lymphoma have changed the way we think about and treat patients with these diseases. She first suggested and demonstrated that adults, including elderly

Speaker Biographies

adults, with acute leukemia could be cured with chemotherapy. She also showed that certain groups of leukemia patients required intensive therapy to be cured, while others could be cured with standard approaches. Her study of chromosomes in leukemia and lymphoma has helped identify genes involved in the development of these diseases, and the application of this information has helped develop effective therapies for individual patients.

Dr. Bloomfield is the recipient of many prestigious honors, including the 2004 American Association for Cancer Research Joseph H. Burchenal Clinical Research Award, 2006 American Society of Clinical Oncology (ASCO) Distinguished Service Award for Scientific Achievement, the 2008 Henry M. Stratton Medal from the American Society of Hematology, ASCO's 2009 David A. Karnofsky Memorial Award, and the 2012 Richard L. Schilsky Cancer and Leukemia Group B (CALGB) Achievement Award.

Michael S. Brown, MD

Michael S. Brown, MD, is Paul J. Thomas Professor of Molecular Genetics and Director of the Jonsson Center for Molecular Genetics at the University of Texas Southwestern Medical School in Dallas. He earned his MD in 1966 from the University of Pennsylvania. He was an intern and resident at the Massachusetts General Hospital, and a post doctoral fellow with Earl Stadtman at the National Institutes of Health. With his colleague, Dr. Joseph L. Goldstein, he discovered the low density lipoprotein (LDL) receptor, which controls cholesterol in blood and in cells. They showed that mutations in this receptor cause Familial Hypercholesterolemia, a disorder that leads to premature heart attacks. Their work laid the groundwork for drugs called statins that block cholesterol synthesis, increase LDL receptors, lower blood cholesterol and prevent heart attacks. Statins are taken daily by more than 20 million people worldwide. Brown and Goldstein shared many awards for this work, including the U.S. National Medal of Science and the Nobel Prize for Medicine or Physiology. Dr. Brown served for 16 years on the Board of Directors of Pfizer, and he is currently a director of Regeneron Pharmaceuticals.

Jean-Laurent Casanova, MD, PhD

Jean-Laurent Casanova, MD, PhD, is the recipient of the 2016 American Society for Clinical Investigation (ASCI) Stanley J. Korsmeyer Award in recognition for his discovery that single-gene inborn errors of immunity can underlie life-threatening infectious diseases in otherwise healthy children and young adults.

Dr. Casanova received his MD in 1987 from Paris Descartes University, and completed his pediatric residency in 1995 and pediatric hematology-immunology fellowship in 1999 at the Necker Hospital for Sick Children and Paris Descartes University. Dr. Casanova also received a PhD in immunology from Pierre et Marie Curie University in 1992. In 1999, he became Professor of Pediatrics at the Necker Hospital and Paris Descartes University, where he is currently a Visiting Professor. He joined the Rockefeller University as a professor in 2008 and was named an investigator of the Howard Hughes Medical Institute in 2014.

Dr. Casanova was elected to the American Society for Clinical Investigation in 2008, the National Academy of Sciences in 2015, and the National Academy of Medicine in 2015. He is a recipient of several prestigious international awards, including the InBev-Baillet Latour Health Prize (Belgium) in 2011 and the Robert Koch Award (Germany) in 2014.

Daniel Drucker, MD

Daniel Drucker, MD, is a Senior Scientist at the Lunenfeld-Tanenbaum Research Institute of the Mount Sinai Hospital, and Professor of Medicine at the University of Toronto. His laboratory studies gut hormone action with a focus on glucagon-like peptide (GLP) hormones. His laboratory has successfully translated insights from basic science findings into new treatments for metabolic disorders. Among these innovations, Dr. Drucker¹s lab discovered the actions of GLP-2 and generated and characterized teduglutide, a GLP-2 receptor agonist now used for the treatment of short bowel syndrome. His studies of incretin action supported the development of two new classes of therapies, DPP-4 inhibitors and GLP-1 receptor agonists, for type 2 diabetes and obesity. Dr. Drucker received his MD from the University of Toronto in 1980 and trained in internal medicine and endocrinology at The Johns Hopkins Hospital, Toronto General Hospital, and Massachusetts General Hospital.

He was recently elected a fellow of the Royal Society and an officer of the Order of Canada.

Gregory Dumanian, MD

Gregory Dumanian, MD, is Chief of Plastic Surgery at Northwestern University Feinberg School of Medicine. As a resident many years ago, Dr. Dumanian experienced numerous finger cuts while tying sutures under tension. He connected the problem of sutures acting as a cheese cutter with the hundreds of thousands of incisional hernias repaired in the United States each year. He recently was awarded a patent for a "mesh suture" that resists the cutting of newly approximated tissues. The suture holds the tissue, and the tissue grows into the suture to improve the strength of the repair. Preclinical animal models and even some human data using strips of mesh as sutures point towards an effective means to appose tissue under tension and to improve on a 3000-year-old medical device.

Anthony S. Fauci, MD

Anthony S. Fauci, MD, is director of the National Institute of Allergy and Infectious Diseases (NIAID) at the U.S. National Institutes of Health, where he oversees an extensive research portfolio devoted to preventing, diagnosing, and treating infectious and immune-mediated diseases. Dr. Fauci also serves as one of the key advisors to the White House and Department of Health and Human Services on global AIDS issues, and on initiatives to bolster medical and public health preparedness against emerging infectious disease threats such as pandemic

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influenza. He was one of the principal architects of the President's Emergency Plan for AIDS Relief (PEPFAR), which has helped save millions of lives throughout the developing world.

Dr. Fauci has made seminal contributions to the understanding of how HIV destroys the body's defenses leading to its susceptibility to deadly infections. Further, he has been instrumental in developing highly effective strategies for the therapy of patients living with HIV/AIDS, as well as for a vaccine to prevent HIV infection.

Dr. Fauci is also the long-time chief of the NIAID Laboratory of Immunoregulation. He has made many contributions to basic and clinical research on the pathogenesis and treatment of immune-mediated and infectious diseases. He is widely recognized for delineating the precise mechanisms whereby immunosuppressive agents modulate the human immune response.

Dr. Fauci is a member of the U.S. National Academy of Sciences and is the recipient of numerous prestigious awards for his scientific and global health accomplishments, including the National Medal of Science, the Robert Koch Medal, the Mary Woodard Lasker Award for Public Service, and the Presidential Medal of Freedom. He has been awarded 42 honorary doctoral degrees and is the author, coauthor, or editor of more than 1,270 scientific publications, including several major textbooks.

Jeffrey M. Friedman, MD, PhD

Jeffrey M. Friedman, MD, PhD, of The Rockefeller University, New York City, is the 2016 recipient of the third annual Harrington Prize for Innovation in Medicine. Dr. Friedman's discovery of leptin and the neural pathway that leptin mediates revealed the underlying mechanism of what drives feeding behavior in mice and humans. Of particular note, his discovery provided a foundation for deeper understanding of related pathologies, such as obesity – a major public-health problem at the time of this discovery in 1994 and persists around the world today.

Dr. Friedman received his MD from Albany Medical College in 1977 and his PhD from The Rockefeller University in 1984. He is the Marilyn M. Simpson Professor at The Rockfeller University and has been an Investigator of the Howard Hughes Medical Institute since 1996.

He is an elected member to the prestigious National Academy of Sciences and is recipient of numerous awards and honors including the Albert Lasker Basic Medical Research Award, the Shaw Prize for Life Sciences and Medicine, and the King Faisal International Prize in Medicine.

Levi Garraway, MD, PhD

Levi Garraway, MD, PhD, is an institute member of the Broad Institute, and is the inaugural director of the Joint Center for Cancer Precision Medicine (CCPM) at the Dana-Farber Cancer Institute, Brigham and Women's Hospital, and the Broad Institute. The overall aim of Dr. Garraway's research is to develop systematic approaches to link genomic changes in tumors to novel avenues for targeted cancer treatments. He has made seminal research contributions in cancer genomics and drug resistance. He published the first genome sequencing studies of aggressive primary prostate cancer, and has led major sequencing initiatives in melanoma and head/neck cancers. In addition to his roles at the Broad, Dr. Garraway is an associate professor of medicine in the Department of Medical Oncology at the Dana-Farber Cancer Institute and Harvard Medical School.

Dr. Garraway has been the recipient of several awards and honors, including the Paul Marks Prize for Cancer Research, the Minority Scholar Award from the American Association of Cancer Research, the Career Award in the Biomedical Sciences from the Burroughs-Wellcome Fund, and the prestigious New Innovator Award from the National Institutes of Health. In 2009, he was inducted into the American Society for Clinical Investigation.

Dr. Garraway received his A.B. in biochemical sciences from Harvard College, and his MD and PhD from Harvard Medical School. He completed his internship and residency in internal medicine at the Massachusetts General Hospital and his fellowship training in medical oncology at the Dana-Farber Cancer Institute.

Joseph L. Goldstein, MD

Joseph L. Goldstein, MD, is currently Regental Professor and Chairman of the Department of Molecular Genetics at the University of Texas Southwestern Medical Center at Dallas.

Dr. Goldstein and his colleague, Michael S. Brown, discovered the low density lipoprotein (LDL) receptor and worked out how these receptors control cholesterol homeostasis. At the basic level, this work opened the field of receptor-mediated endocytosis, and at the clinical level it helped lay the conceptual groundwork for development of drugs called statins that lower blood LDL-cholesterol and prevent heart attacks. Drs. Goldstein and Brown shared many awards for this work, including the Lasker Award in Basic Medical Research (1985), Nobel Prize in Physiology or Medicine (1985), and National Medal of Science (1988).

In recent work, Drs. Goldstein and Brown discovered the SREBP family of transcription factors and showed how these membrane-bound molecules control the synthesis of cholesterol and fatty acids through a newly described process of Regulated Intramembrane Proteolysis. For this work, Drs. Brown and Goldstein received the Albany Medical Center Prize in Medicine and Biomedical Research (2003).

Dr. Goldstein is currently Chairman of the Albert Lasker Medical Research Awards Jury and is a member of the Boards of Trustees of the Howard Hughes Medical Institute and The Rockefeller University. He also serves on the Scientific Advisory Boards of the Broad Institute and Memorial Sloan-Kettering Cancer Center. He is a member of the U.S. National Academy of Sciences and a Foreign Member of the Royal Society.

In addition to his academic activities, Dr. Goldstein serves as Chairman of the Research Advisory Board of GlaxoSmithKline, Inc., and is a member of the Board of Directors of Regeneron Pharmaceuticals, Inc.

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Mehra Golshan, MD

Mehra Golshan, MD, serves as the Medical Director of International Oncology Programs at the Dana-Farber/Brigham and Women's Cancer Center and is the incumbent Dr. Abdul Mohsen and Sultana Al-Tuwaijri Distinguished Chair in Surgical Oncology at the Brigham and Women's Hospital. Dr. Golshan is also the Director of Breast Surgical Services at the Dana-Farber/Brigham and Women's Cancer Center and an Associate Professor of Surgery at Harvard Medical School. Dr. Golshan helps coordinate international oncology relations at DFBWCC with institutions around the world. Dr. Golshan leads one of the world's largest breast surgery programs of 11 full-time breast surgeons operating on nearly 2,800 women a year, and organizing the clinical and research efforts of the breast surgeons.

Dr. Golshan's research interest is focused primarily on neoadjuvant therapies for breast cancer and the use of novel imaging and operative technologies targeting breast carcinoma. The focus of the preoperative therapy trials is to target treatment for women with cancer and develop genotypic profiles that will in the future lead to individualized tailored therapies for women with breast cancer. His research is also currently trying to determine if more accurate tumor assessment with breast MRI prior to surgery will lead to a decrease in the number of operative procedures required to achieve clear margins.

Diane E. Griffin MD, PhD

Diane E. Griffin MD, PhD, is University Distinguished Service Professor and former Chair of Molecular Microbiology and Immunology at the Johns Hopkins Bloomberg School of Public Health and Vice President of the U.S. National Academy of Sciences. Her research interests are in the area of pathogenesis of viral diseases with a particular focus on measles and alphavirus encephalitis. These studies address issues related to virulence and the role of immune responses in protection from infection and in clearance of infection.

She earned her BA in Biology at Augustana College in Rock Island, IL and her MD and PhD at Stanford University School of Medicine.

Dr. Griffin is past president of the American Society for Virology and the American Society for Microbiology. Among other honors, she has received the Rudolf Virchow Medal (2010), Wallace Sterling Lifetime Alumni Achievement Award from Stanford University (2011), the FASEB Excellence in Science Award (2015), and Maxwell Finland Award (2016).

Christopher Hsu, MD, PhD

Christopher Hsu, MD, PhD, is currently finishing his Epidemic Intelligence Service (EIS) assignment in the Division of High-Consequence Pathogens, Poxvirus and Rabies Branch, at the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia. He was one of the first CDC staff to be directly involved with the 2014 Ebola epidemic in West and Central Africa having been involved with the U.S. domestic response, infections in Sierra Leone and the separate but concurrent Ebola outbreak in the Democratic Republic of Congo. He has also been involved in outbreak investigations of chikungunya, poxvirus and rabies throughout the world. His major interests are zoonotic infections in international health settings, particularly the clinical impacts of human rabies from bats and the ecology and prevention of poxvirus infections in Africa, as well as novel pathogen discovery. He will be staying at the poxvirus and rabies branch after his EIS assignment is complete in June 2016. He is currently a medical officer as a Lieutenant Commander in the U.S. Public Health Service.

Dr. Hsu received his BA at the University of Pennsylvania and his master's in public health at Emory University. He then enrolled in the MD/PhD Medical Scholars program at the University of Illinois in Champaign, where his PhD was in veterinary pathobiology, studying interspecies transmission of a herpesvirus. He completed his residency in general pediatrics at the University of Chicago and a fellowship in oncology and post-doctoral research fellowship at Stanford University.

John P.A. Ioannidis, MD

John P.A. Ioannidis, MD, DSc, holds the C.F. Rehnborg Chair in Disease Prevention at Stanford University. His other roles at Stanford include Professor of Medicine, and of Health Research and Policy, and director of the Stanford Prevention Research Center at the School of Medicine; Professor of Statistics (by courtesy) at the School of Humanities and Sciences; one of two directors of the Meta-Research Innovation Center at Stanford; and director of the PhD program in Epidemiology and Clinical Research. His PLoS Medicine paper on "Why Most Published Research Findings are False," has been the most-accessed article in the history of Public Library of Science (exceeding 1.5 million hits). His current citation rate (>1,600 new citations per month per Google Scholar, >800 new citations per month per Scopus or Web of Knowledge) places him in the 100 most-cited among all 20+ million authors publishing across science. Dr. Ioannidis considers himself privileged to have learned and to continue to learn from interactions with students and young scientists (of all ages) from all over the world and to be constantly reminded that he knows next to nothing.

Sarah Jackson, PhD

Sarah Jackson, PhD, is the executive editor for both the *Journal of Clinical Investigation* and *JCI Insight*, a new journal of the American Society for Clinical Investigation (ASCI). As executive editor, she works closely with the Editorial Board of the *JCI* to ensure quality and timely evaluation of research submissions. She also collaborates with the Editor in Chief to direct journal policies and oversee fraud investigations at both journals. Previously, Dr. Jackson worked in grants administration at the North Carolina Biotechnology Center. She received her PhD in biochemistry from the University of North Carolina at Chapel Hill for her research in Yue Xiong's lab on the function of a cullin-based ubiquitin ligase complex in cell cycle progression and its role *in vivo* in B and T lymphocyte development.

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Robert Langer, PhD

Robert Langer, PhD, is an Institute Professor at MIT. His h-index of 211 is the highest of any engineer in history and he has more than 1,080 issued and pending patents worldwide. His patents have licensed or sublicensed to more than 300 companies. He served as Chairman of the FDA's Science Board (the FDA's highest advisory board) from 1999-2002. Langer is also one of very few individuals ever elected to the Institute of Medicine of the National Academy of Sciences, the National Academy of Engineering, the National Academy of Sciences and the National Academy of Inventors. He is one of four living individuals to have receive both the United States National Medal of Science and the United States National Medal of Technology and Innovation. In 2015, Dr. Langer received the Queen Elizabeth Prize for Engineering. He has also received the Charles Stark Draper Prize (considered the engineering Nobel Prize), Albany Medical Center Prize, the Wolf Prize for Chemistry, the Millennium Technology Prize, the Priestley Medal (highest award of the American Chemical Society), the Gairdner Prize, the Kyoto Prize and the Lemelson-MIT prize, for being "one of history's most prolific inventors in medicine." He holds 25 honorary doctorates including honorary degrees from Harvard and Yale.

Andrew Lee, PhD

Andrew Lee, MD, PhD, is the co-founder of StartX Med, the official Medical Innovation Accelerator of Stanford University and Stanford Healthcare. He is also a founder and managing director of StartX-QB3 Labs, a joint medical science and engineering venture between Stanford's StartX and UCSF's QB3 programs. Prior to joining StartX, Andrew was the founding CEO for Stem Cell Theranostics, a venture-backed biotechnology spinout from the Stanford Cardiovascular Institute. As an MD/PhD candidate, Andrew has published more than 50 peer reviewed research manuscripts on the use of medical technologies in treatment of heart and bone disease. His research has been supported by the Howard Hughes Medical Institute, the FDA, and the American Heart Association as well as several of the top 10 global pharmaceutical companies.

Parisa Lotfi, MD

Parisa Lotfi, MD, is a Harvard radiologist that currently focuses her practice on breast imaging at the Brigham and Women's Hospital. She also serves a consultant at the Dana-Farber Cancer Institute. She has been an attending radiologist at the Harvard affiliated hospitals for over 12 years. She currently serves on the Medical Staff Executive Committee at Brigham and Women's Faulkner Hospital. She dedicates her life to the clinical screening, diagnosis and advanced imaging and development for breast cancer and benign breast disease. Her expertise include digital mammography, tomosynthesis, breast MRI, breast ultrasound, PET/CT imaging and breast ultrasound. There are more than 1.7 million cases of breast cancer each year and the clinical screening, diagnostic and biopsy modalities are the cornerstone for the management of this disease process; Dr. Lotfi is a clinical expert in all of these areas. She has lectured across the globe on various advancements in breast imaging.

Dr. Lotfi completed her undergraduate degree at George Washington University and earned her MD from Medical College of Virginia. She conducted her radiology residency at University of Illinois/Michael Reese Hospital following a one year transitional internship at the University of Chicago. She completed a body/abdominal fellowship at Northwestern/ Evanston Hospital in 2001.

Helen Mayberg, MD

Helen Mayberg, MD, is Professor of Psychiatry, Neurology and Radiology and the Dorothy Fuqua Chair in Psychiatric Neuroimaging and Therapeutics at Emory University. Over the last 25 years, her multi-disciplinary depression research team has worked to integrate cutting-edge imaging strategies, quantitative behavioral and psychophysiological metrics, and experimental treatment trials to define brain-based biomarkers that can optimize treatment selection for individual patients. This work was foundational for the first studies of subcallosal cingulate deep brain stimulation for treatment resistant depression and remains the cornerstone of current studies to both refine and optimize DBS implementation and characterize network mechanisms mediating its antidepressant effects.

Dr. Mayberg is a neurologist, trained at Columbia's Neurological Institute in New York, with fellowship training in nuclear medicine at Johns Hopkins. She is a member of the National Academy of Medicine, among other honors, and participates in a wide variety of editorial, advisory and scientific activities across multiple fields in neuroscience.

Carrie Mendoza, MD

Carrie Mendoza, MD, FACEP, is a native Chicagoan with a love of both art and science. She attended Tufts University and followed her interest in art, receiving a master's degree in art history at The University of Chicago. After working at the Art Institute of Chicago, she returned to The University of Chicago for medical school to fulfill her lifelong dream of becoming a doctor. She completed an Emergency Medicine residency at Denver Health Medical Center and a fellowship in Medical Toxicology at the Rocky Mountain Poison and Drug Center. After living and working in Denver for 11 years in a community hospital system, she returned to Chicago with her husband and three sons. She currently works as an Attending Emergency Medicine physician at Advocate Illinois Masonic Medical Center, a Level 1 Trauma Center and training center for University of Illinois at Chicago Emergency Medicine residents on the north side of Chicago. Dr. Mendoza joined MATTER, Chicago's new medical technology incubator, at its opening to launch her startup, Central Line Health, a company dedicated to using creativity and technology to renew and restore the doctor-patient relationship. She developed a communications platform for emergency departments to automate updates, create emergency care specific content, and better manage the waiting experience. By aligning emergency department physician-to-patient communication with the 21st century mobile revolution, the patient and physician experiences are improved and hospital revenue is increased.

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Andy Minn, MD, PhD

Andy Minn's laboratory is focused on understanding how cancer acquires treatment resistance to both conventional therapies and to immunotherapies, and how resistance can be overcome. In order to better understand the basis for this, his lab utilizes data-driven genomics approaches towards both experimental and translational research goals. Hypothesis generation and testing relies on integrating various data types from animal models, molecular biology, genome-wide profiling, immune profiling, clinical data mining, and statistical modeling. Using these methods his lab has identified regulatory networks and signaling pathways that not only predict but also promote treatment resistance. They have discovered that signaling pathways that are normally associated with an anti-viral response are associated with treatment resistance, suggesting an intriguing overlap between the anti-viral response and ways that cancer can evade the cytotoxic effects of therapy and/or the immune system. Dr. Minn's lab is currently investigating this overlap by studying the regulation by the tumor microenvironment and how these anti-viral responses influence response to therapy. The labe is also investigating how tumor response to immune checkpoint blockade can be enhanced with ablative tumor radiation, how resistance to this combination therapy can develop, and how resistance can be therapeutically reversed. Studies using mouse models have been performed in parallel with analogous clinical trials in order to corroborate pre-clinical results with human patients. In this way, laboratory findings can be used to inform the design of next generation clinical trials.

Gary J. Nabel, MD, PhD

Gary J. Nabel MD, PhD, is Chief Scientific Officer for Global Research and Development at Sanofi. He also serves as a Senior Vice President and Deputy to the President for Global R&D and chairs the Strategic Development and Scientific Advisory Council and Ebola Response Coordination Team.

Dr. Gary Nabel joined Sanofi in 2012 from the National Institutes of Health, where he served as director of the Vaccine Research Center (VRC) of the National Institute of Allergy and Infectious Diseases. During his tenure at the NIH, Dr. Nabel provided overall direction and scientific leadership of the basic, clinical, and translational research activities of the VRC.

Dr. Nabel graduated magna cum laude from Harvard College and continued his graduate studies at Harvard, completing his PhD and his MD. He then served as a postdoctoral fellow in the laboratory of David Baltimore at MIT's Whitehead Institute. Dr. Nabel subsequently served as the Henry Sewall Professor of Internal Medicine, professor of biochemistry, and Howard Hughes Medical Institute investigator at the University of Michigan. There, Dr. Nabel also served as the director of the Center for Gene Therapy and co-director of the Center for Molecular Medicine.

Dr. Nabel was elected to the Institute of Medicine of the National Academy of Sciences in 1998. Among his many other honors, Dr. Nabel received the Amgen Scientific Achievement Award from the American Society for Biochemistry and Molecular Biology, the Health and Human Services Secretary's Award for Distinguished Service, and is a fellow of the American Association of Physicians, and the American Academy of Arts Sciences.

Stuart H. Orkin, MD

Stuart H. Orkin, MD, serves as Chairman of the Department of Pediatric Oncology at the Dana-Farber Cancer Institute, the David G. Nathan Professor of Pediatrics at Harvard Medical School, and an Investigator of the Howard Hughes Medical Institute. In addition, he is a principal faculty member of the Harvard Stem Cell Institute and an associate of the Broad Institute of MIT/Harvard. His research focuses on intersections of transcriptional control with stem cell biology, hematopoiesis, and cancer. His research achievements include comprehensive dissection of the molecular basis of the thalassemia syndromes, positional cloning of the first human disease gene, cloning of the first master regulator of blood cell development, and identification of a major silencer of fetal hemoglobin expression.

He received his BS from MIT and MD from Harvard Medical School. He completed pediatric hematology/oncology training at Boston Children's Hospital and the Dana-Farber Cancer Institute, and trained in the laboratory of Philip Leder at NIH.

Dr. Orkin served on the National Research Council Committee on Mapping the Human Genome and as co-chair (with Arno Motulsky) of the Panel to Assess the NIH Investment in Gene Therapy. He was the inaugural chair of the Grants Reviews Committee of the California Institute of Regenerative Medicine.

He is also an elected member of the National Academy of Sciences, Institute of Medicine, and American Academy of Arts and Sciences, and recipient of the E. Mead Johnson Award of the American Academy of Pediatrics, the Warren Alpert Prize, the Helmut Horten Foundation Prize, the Distinguished Research Award from the Association of American Medical Colleges, the E. Donnall Thomas, Dameshek and Basic Science Mentor Awards of the American Society of Hematology, and the Metcalf Award of the International Society of Experimental Hematology. In 2013 he received the Jessie Stevenson Kovalenko Medal of the NAS for "important contributions to the medical sciences." In 2014 he was recognized with the William A. Allan Award from the American Society of Human Genetics.

Stephen Ostroff, MD

Stephen Ostroff, MD, has been FDA's Acting Commissioner since April 2015. Dr. Ostroff joined FDA in 2013 as Chief Medical Officer in the Center for Food Safety and Applied Nutrition and Senior Public Health Advisor to FDA's Office of Foods and Veterinary Medicine. He previously served as FDA's Chief Scientist, where he was responsible for leading and coordinating FDA's cross-cutting scientific and public health efforts. The Office of the Chief Scientist works closely with FDA's product centers, providing strategic leadership and support for FDA's regulatory science and innovation initiatives.

Prior to that, he served as Deputy Director of the National Center for Infectious Diseases at the Centers for Disease Control and Prevention. He retired from the Commissioned Corps of the U.S. Public Health Service at the rank of Rear Admiral (Assistant Surgeon General).

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Dr. Ostroff was the Director of the Bureau of Epidemiology and Acting Physician General for the Commonwealth of Pennsylvania and has consulted for the World Bank on public health projects in South Asia and Latin America.

Dr. Ostroff graduated from the University of Pennsylvania School of Medicine in 1981 and completed residencies in internal medicine at the University of Colorado Health Sciences Center and preventive medicine at CDC. He is a fellow of the Infectious Disease Society of America and the American College of Physicians, and prior to assuming the role of FDA's Acting Commissioner, he chaired the Public Health Committee of the American Society for Microbiology's Public and Scientific Affairs Board.

Anna Penn, MD, PhD

Anna Penn, MD, PhD, is a clinical neonatologist and developmental neuroscientist at Children's National Health System in Washington DC. She received her MD and PhD from Stanford (MSTP), did her residency at University of California San Francisco and returned to Stanford for fellowship and later joined the faculty. Recently, she was recruited to Children's National where she is in the Fetal Medicine Institute, with additional appointments in the Division of Neonatology, the Center for Neuroscience Research and Associate Professor of Pediatrics at George Washington University. She is the director of translational research for Hospital Based Specialties and Director of the Board of Visitors Cerebral Palsy Prevention Program, a new multidisciplinary program at Children's National aimed at improving neurological outcome in preterm infants while training the next generation of cerebral palsy investigators. In her laboratory, Dr. Penn conducts translational work aimed at understanding and ameliorating preterm brain injury. Specifically, she studies the role of placental function in fetal brain development and damage, with the goal of developing new therapeutic agents to protect the brain in sick newborns.

Sallie Robey Permar, MD, PhD

Sallie Robey Permar, MD, PhD, is a physician scientist focusing on the prevention and treatment of neonatal viral infections. She leads a research laboratory investigating immune protection against vertical transmission of neonatal viral pathogens, namely HIV and cytomegalovirus (CMV), using human cohorts and nonhuman primate models. Dr. Permar has made important contributions to the development of vaccines for prevention of vertical HIV transmission, defining both innate and adaptive immune responses that are associated with protection against infant HIV acquisition. Moreover, Dr. Permar is leading the development of HIV vaccine strategies in maternal/infant nonhuman primate models and clinical vaccine trials in infants. She has also contributed to understanding the immunology of perinatal CMV transmission and the pathogenesis of postnatal infection in preterm infants. Dr. Permar developed the nonhuman primate model of congenital CMV infection and uses this model for defining the immune correlates of protection against CMV transmission and vaccine development.

Jakeb Riggle, PhD

Jakeb Riggle, PhD, is in his final year of an MD/PhD dualdegree program at the University of Nebraska Medical Center (UNMC). Born and raised near Omaha, Nebraska, he completed his bachelor of science degree in biosystems engineering from the University of Nebraska-Lincoln in 2008. Dr. Riggle completed a PhD in biomedical engineering at UNMC in 2014, with a focus on laparoscopic surgical device design and simulation training of physician trainees. He is coinventor on a U.S. and international patent for a laparoscopic surgical instrument currently being licensed for further development. Dr. Riggle hopes to continue his research and engineering development during his upcoming residency in internal medicine.

Maryam Saleh, PhD

Maryam Saleh, PhD, is passionate about moving ideas into the marketplace, where it can have impact and improve lives. She is a computational neuroscientist (earning her PhD from the University of Chicago) and computer engineer (BSc from Brown University). While earning her degrees, she joined Cyberkinetics Neurotechnology Systems Inc., to launch a brain implant that promised to restore movement in paralyzed individuals. After completing her doctorate, she grew increasingly interested in startups and joined Northwestern's tech transfer office where she founded the Center for Device Development, a fellowship program where doctors and engineers test drive their medical device business idea. In September 2014, she joined MATTER's launch team. Her goal as VP of Programs at MATTER is to find meaningful ways to connect entrepreneurs, payers, providers, investors, and manufacturers to accelerate healthcare innovation.

Larry Schlesinger, MD

Larry Schlesinger, MD, is the Samuel Saslaw Professor of Medicine and chair of the department of Microbial Infection and Immunity. He directs the Oregon State University (OSU) Center for Microbial Interface Biology, an interdisciplinary campus-wide program that focuses on infectious diseases of major concern to human health. He is an internationally recognized physician scientist in the pathogenesis of tuberculosis and other diseases due to intracellular pathogens that subvert lung immune mechanisms. He has devoted his entire career to better understanding the human innate immune response to pathogens and translates his discoveries into drug discovery platforms. He was named to the inaugural class of the Harrington Scholar-Innovator Award for drug discovery and is pursuing new TB treatments. He is currently an NIH Council member, Fellow of the AAAS and American Academy of Microbiology, and OSU's 2011 Distinguished Scholar.

Dr. Schlesinger has placed great emphasis on education and mentoring throughout his career, particularly in translational and clinical research. He is currently PI of 2 NIH T32 training grants. In all, he has mentored more than 130 trainees at all levels, several of whom have been awarded national research fellowships (33 in total) and have gone on to academic or industry positions. He was awarded the first ever OSU NIH-

Speaker Biographies

funded MSTP in 2011 and serves on the MD/PhD subsection steering committee of the AAMC.

Julie Segre, PhD

Julie Segre, PhD, is a Senior Investigator at the National Human Genome Research Institute, NIH. Her research focuses on microbial genomics, investigating both hospital pathogens and the diversity of commensal skin organisms. Dr. Segre's research integrates DNA sequence technology, algorithm development and diagnostic clinical microbiology. Segre has published extensively with 30 research articles (19 as senior author) and five review articles in the last five years in journals including *Science, Nature, Science Translational Medicine, PNAS, Genome Biology* and *Genome Research*.

Dr. Segre's research has defined the normal human skin bacterial and fungal communities, enabling studies of alterations associated with pediatric atopic dermatitis and primary immunodeficiency. Segre's research also focused on integrating whole genome sequencing of hospital pathogens both to study nosocomial transmission and to develop a national surveillance network.

Segre received the 2013 Service to America Medal, together with NIH Clinical Center epidemiologist Tara Palmore, for deploying genomic sequencing to guide hospital outbreak containment. Segre received her BA from Amherst College in 1987, graduating Phi Beta Kappa and summa cum laude in mathematics. She obtained her PhD in 1996 in Genetics from MIT, advised by Eric S. Lander, PhD, and received postdoctoral training in skin biology mentored by Elaine Fuchs, PhD. Segre was recruited as a new investigator to NHGRI/NIH in 2000 and received tenure in 2007. Segre was elected to the Board of Trustees of Amherst College in 2011.

Christine E. Seidman, MD

Christine Seidman, MD, is the Thomas W. Smith Professor of Medicine and Genetics at Harvard Medical School and Brigham and Women's Hospital and an Investigator of the Howard Hughes Medical Institute. Her laboratory uses genomic strategies to define causes of human cardiovascular disease, including congenital heart malformations and cardiomyopathies. By exploiting model systems to identify pathways impacted by mutations, these studies have enabled gene-based diagnostics and novel strategies to limit the deleterious consequences of human mutations. Dr. Seidman also leads multi-institution consortium that assess rare and common variants involved in cardiovascular phenotypes and that explore the clinical utility of genomic variation in early diagnosis and prevention of cardiovascular disease.

She was an undergraduate at Harvard College and received a MD from George Washington University School of Medicine. After clinical training in internal medicine at John Hopkins Hospital she received subspecialty training in cardiology at the Massachusetts General Hospital. Dr. Seidman is a faculty member of Brigham and Women's Hospital, where she serves as director of the Brigham Research Institute. She is the founding director of the BWH Cardiovascular Genetics Center.

The recipient of many honors, Dr. Seidman is a Distinguished Scientist of the American Heart Association, Fellow of the American Academy of Arts and Sciences, and member of the Institutes of Medicine and the National Academy of Sciences.

Jonathan G. Seidman, PhD

Jonathan Seidman, PhD, is the Henrietta B. and Frederick H. Bugher Professor of Cardiovascular Genetics at Harvard Medical School. He received his undergraduate degree from Harvard University and his PhD from the University of Wisconsin-Madison. His postdoctoral studies were carried out in Dr. Philip Leder's laboratory at the National Institute of Child Health and Human Development. He has been a member of the Genetics Department, Harvard Medical School since 1981.

The Seidman Laboratory, which Jonathan co-runs with his wife Christine Seidman, MD, studies the genetic basis for human disease. The laboratory's principle focus is defining the genetic contribution to both adult and pediatric cardiovascular disease. Investigations range from the discovery of genetic variants in rare and common cardiovascular phenotypes to elucidation of how genetic variations alter signaling mechanisms in model organisms. To advance these efforts, the laboratory applies high-throughput genomic sequencing for basic investigations and clinical application.

Dr. Seidman is a member of The Genetics Society of America and the American Society of Human Genetics. He has received several awards including the 12th Annual Bristol-Myers Squibb Award for Distinguished Achievement in Cardiovascular Research (2002), jointly with Christine Seidman, MD; the Lefoulon-Delalande Foundation Grand Prix for Science (2007), joint recipient with Christine Seidman, MD; the Katz Prize for Cardiovascular Research awarded by Columbia University School of Medicine (2008), jointly with Christine Seidman, MD; the Distinguished Scientist Award from the American Heart Association (2013) and the Sarnoff Cardiovascular Research Foundation Mentorship Award (2014). He is also a member of the National Academy of Science (2007) and the Institutes of Medicine (2007).

Michael Snyder, MD, PhD

Michael Snyder, MD, PhD, is the Stanford Ascherman Professor and Chair of Genetics and the director of the Center of Genomics and Personalized Medicine. He is a leader in the field of functional genomics and proteomics, and one of the major participants of the ENCODE project. His laboratory study was the first to perform a large-scale functional genomics project in any organism, and has developed many technologies in genomics and proteomics. These include the development of proteome chips, high resolution tiling arrays for the entire human genome, methods for global mapping of transcription factor binding sites (ChIP-chip now replaced by ChIP-seq), paired-end sequencing for mapping of structural variation in eukaryotes, de novo genome sequencing of genomes using high throughput technologies and RNA-Seq. Seminal findings from the Snyder laboratory include the discovery that much more of the human

Speaker Biographies

genome is transcribed and contains regulatory information than was previously appreciated, and a high diversity of transcription factor binding occurs both between and within species. He has also combined different state-of-the-art "omics" technologies to perform the first longitudinal detailed integrative personal omics profile (iPOP) of a person and used this to assess disease risk and monitor disease states for personalized medicine.

Dr. Snyder received his PhD training at the California Institute of Technology and carried out postdoctoral training at Stanford University. He is a cofounder of several biotechnology companies, including Protometrix (now part of Life Technologies), Affomix (now part of Illumina), Excelix, and Personalis, and he presently serves on the board of a number of companies.

David Tuveson, MD, PhD

David Tuveson, MD, PhD, is Professor and Deputy Director of the Cancer Center at Cold Spring Harbor Laboratory. Dr. Tuveson trained at MIT (BS, Chemistry, 1987), Johns Hopkins (MD, PhD 1994), Brigham and Women's Hospital (House Officer 1994-7) and Dana-Farber/Harvard (Medical Oncology Fellow 1997-2000). While at Dana-Farber, he co-developed Imatinib with George Demetri for patients with gastrointestinal stromal tumors (GIST). Dr. Tuveson performed post-doctoral research with Tyler Jacks at MIT, where he developed several mouse cancer models and investigated GIST. He was appointed an Assistant Professor of Medicine at the University of Pennsylvania from 2002–2006, where his laboratory developed the first ductal pancreatic cancer models.

Shortly thereafter, Dr. Tuveson was recruited in 2006 to the CRUK/Cambridge Research Institute at the University of Cambridge to establish a preclinical therapeutics laboratory and a pancreatic cancer clinical trials group. In Cambridge, his laboratory determined several mechanisms that contribute to drug resistance in pancreatic cancer, stimulating clinical trials in these areas. He was appointed Professor of Pancreatic Cancer Medicine at the University of Cambridge, and founder of the Pancreatic Cancer Centre.

Dr. Tuveson was recruited to Cold Spring Harbor Laboratory in 2012 to lead the Cancer Therapeutics Initiative. In 2015, Dr. Tuveson co-developed pancreatic cancer organoid models with Dr. Hans Clevers, a new platform for basic discovery and translational medicine. Dr. Tuveson also serves as Director of Research for the Lustgarten Foundation for Pancreatic Cancer Research, and has an appointment in medical oncology at Memorial Sloan Kettering Cancer Center. Past prizes include the Rita Allen Foundation award and the Jan Waldenstrom Award.

David Wright, MD

David Wright, MD, is a tenured associate professor of emergency medicine, director of the Emergency Neurosciences Laboratory in the Department of Emergency Medicine, and the director for the Center for Injury Control at Emory University. He is a board certified emergency medicine physician practicing at Emory affiliated hospitals and Grady Memorial Hospital, Atlanta's primary Level 1 Trauma Center. His interests include the prevention and mitigation of injury, and the preclinical and clinical assessments of traumatic brain injury, stroke and other acute neurological conditions.

He is also an adjunct faculty in the Department of Biomedical Engineering at the Georgia Institute of Technology and works closely with an elite team of engineers at the Georgia Tech Research Institute where he participates in numerous concussion research and technology development endeavors. He is the co-inventor of the DETECT technology, a rapidly deployable, easily administered, comprehensive system for the assessment of concussion and other neurological disorders.

Richard A. Young, PhD

Richard Young, PhD, is a Professor of Biology at the Whitehead Institute and MIT. Dr. Young studies gene control in health and disease and is known for discovering the core regulatory circuitry of embryonic stem cells. He has served as an advisor to the World Health Organization, the National Institutes of Health and numerous scientific societies and journals. Dr. Young has founded and advised companies in the biotechnology and pharmaceutical industry, and is currently a member of the Board of Directors of Syros Pharmaceuticals. His honors include membership in the National Academy of Sciences; *Scientific American* has recognized him as one of the top 50 leaders in science, technology and business. Dr. Young is also an aviator and holds a commercial pilot license.

Speaker Biographies

Jennifer Zeitzer, PhD

Jennifer Zeitzer, PhD, has served as the Director of Legislative Relations at the Federation of American Societies for Experimental Biology (FASEB) since October 2008, and was recently appointed the Deputy Director of FASEB's Office of Public Affairs. In this role, she directs the FASEB Capitol Hill Office, represents FASEB on Capitol Hill, manages FASEB's communications with the U.S. Congress, and develops legislative strategies related to issues involving funding for the National Institutes of Health and other federal science agencies. She also coordinates FASEB's advocacy efforts with other partners in the biomedical and scientific research community and organizes the annual FASEB Capitol Hill Day.

In December 2013, Dr. Zeitzer was re-elected to the Coalition for Health Funding Board of Directors. She is also a member of the Executive Committee of the Friends of VA Medical Care and Health Research (FOVA) Coalition and serves on the Steering Committee of the Ad Hoc Group for Medical Research. Her areas of expertise include the federal budget and appropriations process, IRS regulations concerning lobbying by non-profit organizations, and grassroots advocacy for non-profits.

Prior to joining FASEB, Dr. Zeitzer was at the Alzheimer's Association for 13 years where she served in various capacities, including Director of Congressional Relations. She led the association's efforts in support of federal funding for biomedical research. Additionally, Dr. Zeitzer served as chair of the National Health Council Government Relations Affinity Group from 2005 – 2006. She is a member of the National Academy of Social Insurance and has a bachelor's degree in political science from the Pennsylvania State University.

Huda Zoghbi, MD

Huda Zoghbi, MD, is Professor of Pediatrics, Neurology, Neuroscience, and Molecular and Human Genetics at Baylor College of Medicine and serves as an Investigator with the Howard Hughes Medical Institute. She is also the director of the Jan and Dan Duncan Neurological Research Institute at Texas Children's Hospital.

Dr. Zoghbi's interests range from neurodevelopment to neurodegeneration. Her discovery that Spinocerebellar Ataxia type 1 is caused by expansion of a polyglutamine tract and that such expansion leads to accumulation of the mutant protein in neurons has had profound ramifications since many late-onset neurological disorders involve similar accumulations of disease-driving proteins. Dr. Zoghbi's work in neurodevelopment led to the discovery of the gene Math1/Atoh1 and to showing that it governs the development of several components of the proprioceptive, balance, hearing, vestibular, and breathing pathways. Her group also discovered that mutations in MECP2 cause the neurological disorder Rett syndrome. We now know that mutations in this gene are responsible for a broad spectrum of disorders ranging from mild cognitive disabilities to autism. Her lab is focused on understanding how loss of MeCP2 alters neuronal function to cause behavioral abnormalities.

Dr. Zoghbi trained many scientists and physician-scientists and is a member of several professional organizations and boards. Among her honors are Gruber Prize in Neuroscience, the Pearl Meister Greengard Prize from Rockefeller University, the Scolnick Prize from MIT, and the March of Dimes Prize in Developmental Biology. In 2000 she was elected to the Institute of Medicine, and in 2004 she was elected to the National Academy of Sciences.

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APSA Trainee Oral Presentations

1

Genomic Editing in iPSCs Establishes that a Rare TBX5 Variant causes Brugada Syndrome

K Bersell[1], **T Yang**[1], **C Hong**[1], **E Behr**[2], **E Petropoulou**[2], **Y Jamshidi**[2], **Q Wells**[1], **P Kannankeril**[1], **D Roden**[1]

[1]Vanderbilt University, Nashville, TN; [2]St George's University of London, London, United Kingdom (Great Britain)

Introduction: We have identified a novel missense variant in the transcription factor TBX5 (resulting in G145R) in a kindred with Brugada syndrome (BrS). Reduced sodium current (INa) due to fewer sodium channels at the cardiomyocyte membrane or mutations impairing channel function is a major cellular mechanism of BrS. TBX5 is known to positively regulate expression of the cardiac sodium channel gene SCN5A. In silico modeling predicts G145R is a loss of function allele, and in preliminary studies we found that TBX5-G145R reduced DNA binding by 97% and TBX5 transcriptional activity *in vitro* by 61%. These data support but do not establish a role for TBX5-G145R as a cause of BrS. **Methods:** Dermal fibroblasts from TBX5-G145R carriers diagnosed with BrS and unrelated population control donors were reprogrammed to iPSCs. The TBX5 variant was edited to wild-type using CRISPR/Cas9 technology. iPSCs were differentiated to cardiomyocytes (iPSC-CMs) using chemical modification of the Wnt pathway for transcriptional and electrophysiologic studies. **Results:** TBX5-G145R (BrS) iPSC-CMs had reduced sodium channel transcript (-73%), membrane-associated protein (-48%), and decreased INa (-97.1±36.5 pA/pF; n=8) versus population controls (-211±36.0 pA/pF; n=13, $P<0.05$). After CRISPR/Cas9 editing, sodium channel expression and current density (-198±38 pA/pF; n=10) in iPSC-CMs were restored to population control values. Unexpectedly, TBX5-G145R iPSC-CMs also displayed increased late INa (TBX5-G145R: 2.2±0.4%; Control: 0.16±0.05%; n=5, $P<0.05$), increased action potential durations (APDs; TBX5-G145R: 517±72 msec; Control: 321±37 msec; n=7, $P<0.05$), and early and later afterdepolarizations (EADs/DADs), all reversed by the late INa blocker ranolazine. These arrhythmogenic properties were also reversed after genomic correction of the TBX5 variant. **Conclusions:** These data close the loop between association and causation by proving that TBX5-G145R causes the BrS phenotype of reduced INa and the additional findings of increased late INa, APD prolongation, and EADs/DADs. This approach establishes a new paradigm for studying the pathogenicity of rare variants in congenital arrhythmia syndromes.

2

SRC-2 integrates polygenic inputs to maintain glucose homeostasis

T Fleet[1], **B Zhang**[1], **F Lin**[1], **B Zhu**[1], **S Dasgupta**[1], **K Rajapakshe**[1], **N Timchenko**[2], **C Coarfa**[1], **B York**[1], **B O'Malley**[1]

[1]Baylor College of Medicine, Houston, TX; [2]University of Cincinnati College of Medicine, Cincinnati, OH

Obesity is a growing global epidemic that has increased the preponderance of co-morbidities such as Type II Diabetes Mellitus (T2DM). It is clear that diseases such as T2DM are a result of complex interacting factors including the environment and genetic inputs, but the precipitating events that lead to hyperglycemia and insulin resistance and account for the wide spectrum of clinical presentation have not been identified. Therefore, T2DM is commonly defined as a polygenic disorder in which multiple genes fractionally contribute to the phenotypic outcomes of insulin resistance and eventual sequelae. Peripheral glucose is regulated by the liver, which coordinates gene expression programs for glucose absorption, storage, and secretion. We identified hepatic SRC-2 as a stabilizer of nutritionally responsive transcriptional complexes that modulate peripheral glucose availability. With DNA pull-down followed by mass spectrometry, we defined SRC-2 dependent transcriptional complexes on rate-limiting genes in glycolysis and gluconeogenesis. Our findings suggest a novel model of polygenic disorders in which platform coactivators such as SRC-2 regulate multiple genetic inputs that contribute to the varied presentations of glucose metabolism diseases.

Poster Abstracts

1

Repurposing Tromethamine as an Inhaled Therapy to Treat CF Airway Disease

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In mammalian airways, the composition of the thin layer of fluid that lines the mucosal surface, airway surface liquid (ASL), is critical for the proper function of airway host defenses. In cystic fibrosis (CF), loss of cystic fibrosis transmembrane conductance regulator (CFTR) function causes ASL pH to become acidic, which impairs airway host defenses. One potential therapeutic approach to correct the acidic pH in CF airways is by aerosolizing HCO3- and/or non-bicarbonate buffers. Here, we show that raising ASL pH with inhaled HCO3- increased pH. However, the effect was transient and pH returned to baseline values within 30 minutes. Tromethamine (THAM®) is a buffer with a long half-life used in intravenous formulation to treat metabolic acidosis. We found that the addition of tromethamine, *in vivo*, increased ASL pH for at least 1-2 hours and enhanced bacterial killing. Inhaled hypertonic saline (7% NaCl) is a therapy in people with CF. Hypertonic saline promotes mucus clearance in advanced CF airway disease. High salt inhibits ASL antimicrobial factors. Therefore, we wondered whether tromethamine in combination with hypertonic saline would alkalinize sputum collected from individuals with CF and increase bacterial killing. Tromehtamine alone or in combination with hypertonic saline increased pH and enhanced bacterial. Thus, our findings suggest that bicarbonate-independent inhaled buffers such as tromethamine, when used alone or in combination with hypertonic saline, may offer a therapeutic advantage in CF airway disease.

2

β-arrestin Mediates the Frank-Starling Mechanism of Cardiac Contractility

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Background: The Frank-Starling law of the heart is a physiologic phenomenon that describes an intrinsic property of heart muscle where increased cardiac filling leads to enhanced performance. The mechanistic basis for this phenomenon involves a length dependent enhancement of cardiac myofilament Ca2+ sensitivity. However, the upstream molecular events that link cellular stretch to the length dependent myofilament Ca2+ sensitivity are poorly understood. Since angiotensin II type 1 receptors (AT1Rs) and the adaptor protein β-arrestin have recently been shown to mediate mechanosensitive cellular signaling, we tested the hypothesis that these two proteins are involved in the Frank-Starling law of the heart. **Methods:** The Frank-Starling law of the heart was tested by serial pressure-volume loop analyses on Wild type (n=27), β-arrestin 1 KO (n=15), β-arrestin 2 KO (n=18) and AT1R KO (n=6) mice using a conductance catheter. Each group was given a colloid solution to increase preload in order to measure the relationship between cardiac filling and performance. The effect of pharmacologic AT1R blockade on the Frank-Starling law was tested in a separate cohort of Wild type mice treated with Saline (n=10), the conventional AT1R blocker losartan (n=10) or a β-arrestin biased AT1R ligand TRV120023 (n=6). Myofilament Ca2+ sensitivity was tested in skinned muscle fiber preparations isolated from Wild Type, β-arrestin-1, β-arrestin 2 and AT1R KO mice. **Results:** We found that Wild type mice increased stroke volume by 50-60% with volume loading, while no significant increase in stroke volume was noted in β-arrestin 1, β-arrestin 2, or the AT1R KO mice. Wild Type mice pretreated with losartan were unable to enhance cardiac contractility with volume loading, while treatment with a β-arrestin biased AT1R ligand TRV120023 preserved the increase in contractility with volume loading. Lastly, in skinned muscle fiber preparations, we found length dependent myofilament Ca2+ sensitivity in the β-arrestin 1, β-arrestin 2 and AT1R knockout mice to be impaired. **Conclusions:** Our data uncovers β-arrestin 1, β-arrestin 2 and the AT1R as key regulatory molecules in the Frank-Starling mechanism, which can potentially be targeted therapeutically with β-arrestin biased AT1R ligands.

3

Antibiotic exposure disrupts pulmonary immunity and impairs development of tolerance to inhaled house dust mite

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Introduction: Prevalence of allergic asthma has nearly tripled since 1980. Asthma arises from an inappropriate immune response to allergens such as house dust mite (HDM), but our incomplete picture of asthma's immunologic mechanisms has hampered efforts to reduce disease prevalence. One risk factor for asthma development is exposure to antibiotics (ABX), but our limited understanding of the links between ABX and pulmonary immunity has impaired our ability to effectively utilize ABX while simultaneously minimizing asthma risk. **Methods:** In our mouse model of HDM-induced allergic airway disease, intranasal exposure to HDM produces an analog of human asthma, including airway eosinophilia and leukocytosis. However, continued HDM exposure results in development of tolerance and disease suppression. To evaluate the effect of ABX exposure on tolerance development, we divided mice into two groups (n=5 per group). One group received a mix of vancomycin, neomycin, metronidazole, and ampicillin (1 g/L in drinking water) throughout life. Control (untreated) mice received no ABX. At six weeks of age, mice were exposed to intranasal HDM five days per week for eleven weeks, at which time mice were sacrificed. Lung, broncho-alveolar lavage (BAL), and lung-draining (hilar) lymph node (HLN) cells were isolated for flow cytometric analysis and BAL cellular differentials. Comparisons were made using one-way ANOVA or Mann-Whitney U tests. **Results:** Animals exposed to HDM and ABX had significantly higher proportions

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(9.0% vs 1.4%, $p < 0.01$) and ten-fold higher numbers ($p < 0.001$) of eosinophils in the BAL as compared to HDM-only controls. Leukocyte numbers were ten- to twenty-fold higher in the BAL ($p < 0.05$) and twice as high in the lungs ($p < 0.05$) of animals treated with HDM and ABX as compared to HDM-only controls. HLN cellular analysis revealed that proportions of Foxp3+ regulatory T Cells and CD5+ B Cells, both known to reduce allergic disease severity, were lower in HDM- and ABX-treated mice as compared to HDM-only controls (19.3% vs 20.2%, $p < 0.05$ and 4.9% vs 7.6%, $p < 0.05$, respectively). In the lungs, the proportion of CD5+ B Cells was lower in ABX-treated mice as compared to HDM-only controls (2.9% vs 4.2%, $p < 0.01$). **Conclusions:** These data suggest that the increased risk of developing asthma following ABX exposure may result from disruption of pulmonary immune regulatory cells. Investigation of mechanisms behind ABX-induced regulatory cell disturbances, such as disruption of the host microbiota, are ongoing in our laboratory. Work supported by NIAID (R01 AI-043573 to RST/CMS) and NHLBI (F30 HL-126324 to AJA).

4

Heparanase is a host enzyme required for herpes simplex virus release from cells

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Herpesviruses exemplified by herpes simplex virus-1 (HSV-1) attach to cell surface heparan sulfate (HS) for entry into host cells. During a productive infection, the HS moieties on parent cells can also trap newly exiting viral progenies and inhibit their release. Here we demonstrate that a HS-degrading enzyme of the host, heparanase (HPSE), is upregulated through NF-kB activation and translocated to the cell surface upon HSV-1 infection, resulting in HS removal and facilitated viral release. We also find that knockdown of HPSE *in vivo* inhibits virus shedding after corneal infection, indicating that HPSE is likely important in herpetic disease progression. Our findings suggest that the upregulation of HPSE upon infection serves as a means for newly produced, exiting virions to avoid re-attachment to HS and re-entry into parent cells, thus increasing viral spread. Since many human viruses use HS as an attachment receptor, the HPSE-HS interplay may delineate a common mechanism for virus release.

5

Nitrix Oxide Synthase Modulation in the Treatment of Colorectal Cancer

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The American Cancer Society estimates more than 141,000 new cases of and about 50,000 deaths from colorectal cancer every year. Treatment options include surgery, radiation therapy and targeted therapies such as anti-angiogenics. However, no therapies address the key driving factor of colorectal cancer: inflammation. It is well known that chronic inflammatory conditions such as Crohn's Disease, ulcerative colitis, diabetes, obesity and cigarette smoking all elevate the risk of developing colorectal cancer. One of the hallmarks of chronic inflammation is the elevated levels of reactive oxygen/nitrogen species (ROS/RNS). A primary source of these ROS/RNS is uncoupled Nitric Oxide Synthase. Under non-inflammatory conditions NOS generates Nitric Oxide. However, in an inflammatory environment, such as the oxidative tumor microenvironment, NOS's cofactor tetrahydrobiopterin (BH4) is oxidized to dihydrobiopterin (BH2). NOS bound to BH2 is said to be uncoupled and produces superoxide O2- and peroxynitrite (ONOO-). Previous work in our and other's labs have shown that increased production of ROS/RNS leads to the activation of pro-inflammatory/proliferative molecules such as NFκB, Stat3, β-Catenin and Akt. NOS can be re-coupled by supplementing cells and animals with BH4 or its precursor Sepiapterin (SP). Herein we show that recoupling NOS with SP in HCT116, Caco-2 and HT29 cells, decreased tumor cell proliferation, increased β-Catenin degradation and decreased Akt activity. We also see increased tumor cell death measured by *in vitro* clonogenic assay, as well decreased metabolic uptake in AOM/DSS induced colorectal cancer *in vivo* measured by F18Deoxy-Glucose PET imaging. We believe by recoupling NOS both *in vivo* and *in vitro* we are modulating Wnt signaling via Akt and GSK-3β. Lastly, we conducted studies to determine a mechanistic explanation of how tumor cells maintain a decreased BH4:BH2 ratio.

6

Killing two birds with one stone: a novel and translational site-targeted strategy for inhibiting pathogenic IgM binding and complement deposition after ischemic stroke

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Natural IgM antibodies are components of the innate recognition system of danger-associated molecular patterns (DAMPS) expressed by stressed and dying cells. Ischemic stress leads to the binding of natural IgM and complement activation, resulting in the propagation of inflammation and injury. We previously isolated two natural IgM monoclonal antibodies (mAbs) from un-manipulated mice: B4 that recognizes modified annexin-IV,

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and C2 that recognizes a subset of phospholipids. Both B4 and C2 mAbs specifically recognized ischemic cells and reconstituted cerebral ischemia reperfusion injury in otherwise protected antibody-deficient Rag1-/- mice. Therefore, we developed a novel strategy of site-targeted complement inhibition by fusing a single chain antibody (scFv) derived from the B4-mAb hybridoma to the complement inhibitor Crry. The fusion construct, B4scFv-Crry, specifically targeted ischemic cerebral endothelial cells, competed with IgM binding, and inhibited complement deposition after hypoxia and re-oxygenation *in vitro*. Using an in-vivo model of transient middle cerebral artery occlusion with 60 mins ischemia, a single dose of B4scFv-Crry administered 2 hrs after ischemia, inhibited pathogenic IgM and complement deposition in the ischemic brain. B4scFv-Crry treatment also significantly reduced infarct volume (by 82%, $p<0.01$) and neurological deficit scores (by 52%, $p<0.05$) in both young and aged mice as measured 24 hrs after ischemia. B4scFv alone provided similar levels of improvement in infarct volume (78% reduction, $p<0.01$) and neurological deficit (50% reduction, $p<0.05$) at 24 hrs after ischemia, but only B4scFv-Crry provided protection into the subacute phase (7 days post-ischemia), yielding 65% reduction in infarct volume and 42% and 49% improvement in neurological scoring and corner task, respectively ($p<0.05$). We also demonstrated that B4scFv-Crry was similarly protective in a thromboembolic model of ischemic stroke, and reduced infarct volume from 105+/-9 mm3 in vehicle-treated animals to 20.2 +/- 8 mm3 ($p<0.01$), with simultaneous reduction in neurological deficit scores by 46% ($p<0.05$). Finally, we demonstrated that both B4-scFv and B4scFv-Crry bound specifically within the ischemic core and penumbra of samples obtained from patients who died 24-72 hrs after acute stroke. No binding was observed in sections prepared from normal brain tissue from the same patient, or from age-matched controls. We also demonstrated endogenous IgM and complement deposition in ischemic cores and penumbral regions of human brains. These data indicate a similar DAMP recognition system occurs in the brains of mouse and man, and that B4scFv-Crry has a translational potential as a therapeutic agent to reduce the extent of injury and improve outcomes in stroke patients.

7

Dendrite Regeneration: Investigating a Novel Neuronal Injury Response

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Neurons are polarized cells with axon and dendrite compartments crucial for information signaling functions. Axons and dendrites are susceptible to injury that can leave neurons non-functional if damage is left unrepaired. Since most neurons are not replaced during an organism's lifetime, regeneration of axons and dendrites represents an important neuronal survival strategy. Axon regeneration requires the conserved dual leucine zipper kinase (DLK) signaling pathway and has been studied for over 150 years. In contrast, dendrite regeneration has only recently gained attention. We have recently discovered that Drosophila sensory neurons have a robust mechanism for sensing dendrite injury and responding by dendrite regeneration. We found that the DLK pathway is dispensable for dendrite regeneration, and have found little overlap between the processes of axon and dendrite regeneration. This suggests that dendrite regeneration requires a novel neuronal injury response pathway. We have since focused our efforts on studying dendrite regeneration in Drosophila dorsal dendritic arborization C (ddaC) neurons to better understand the underlying molecular mechanisms, as well as evaluating the functional status of regenerated dendrites. To better understand the molecular mechanisms underlying dendrite regeneration, we performed RNA sequencing on pools of 10 ddaC neurons that had undergone no injury, axon injury, or dendrite injury. By comparing results among these three groups we were able to identify dendrite regeneration associated genes that were upregulated following dendrite injury, but not axon injury. We evaluated the maturity level of regenerated dendrites by looking at their ability to prune during metamorphosis. We found that regenerated dendrites pruned during metamorphosis just like normal uninjured dendrites. Lastly, we studied the ability of dendrite regeneration to restore function to neurons by evaluating the previously characterized nociceptive rolling behavior of larva in response to thermal stimulation. We found that dendrite injury disrupted the nociceptive rolling behavior 2 hours following dendrite injury, but was restored as early as 12 hours following injury when regeneration is in its early stages. Our results provide important insight into the molecular mechanisms of dendrite regeneration that will aid future research focused on determining the significance of dendrite injury in disease situations. Furthermore, our data suggests that dendrite regeneration is capable of restoring dendrites to a level where neuronal function is recovered.

8

Regulation of neuromuscular synaptogenesis and motoneuron survival by a single miRNA

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Evidence is mounting that defective RNA metabolism is central to the pathogenesis of diseases affecting motoneurons (e.g. amyotrophic lateral sclerosis and spinal muscular atrophy). Yet, our understanding of motoneuron-specific gene regulatory pathways is largely limited to those mediated by transcription factors. Investigations into motoneuron-specific, RNA-mediated regulatory pathways (such as those involving miRNAs), may provide novel insights into potential pathogenic mechanisms. We and others have identified a single microRNA (miR-218) that is both highly enriched and abundantly expressed in murine motoneurons. Here, using a combination of RNA sequencing and mouse genetics, we identify novel alternative promoters embedded within the Slit2/3 genes that contribute to miR-218's specific expression in brainstem and spinal motoneurons.

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Our most informative and exciting experiments derive from investigation of miR-218 knockout mice, generated by CRISPR-mediated multiplexed deletions of all four miR-218 alleles. Motoneurons in these mice exhibit dramatic neuromuscular synaptic failure, hyperexcitability, and cellular degeneration – the hallmarks of motoneuron diseases. Without miR-218, mice exhibit flaccid paralysis and neonatal death, demonstrating that this single miRNA is indispensable to motoneuron function and survival. How can a single, small non-coding RNA have such a fundamental importance to motoneuron gene regulation? Gene profiling wild type and knockout motoneurons uncovers an impressive network of hundreds of mRNAs that are under miR-218 mediated repression. Using differential expression and unbiased 3'UTR motif-enrichment analysis, we find that miR-218 target genes are expressed lower in motoneurons versus other subpopulations of spinal and cortical neurons. Moreover, we find that miR-218 doesn't merely reinforce/potentiate target genes' reduced expression (as has been suggested for microRNAs in general), but instead constitutively and independently drives the repression of its target network in motoneurons. This study identifies a previously unappreciated miRNA regulatory network that is unique to motoneurons and is critical for *in vivo* neuromuscular synaptogenesis and motoneuron survival.

9

A role for the PERK branch of the unfolded protein response in melanocyte survival

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Background: Cytoprotective stress responses ensure survival of pigment-producing melanocytes in the skin where they are continuously subjected to environmental toxins and UV radiation. We previously demonstrated that chemical-induced endoplasmic reticulum (ER) stress or oxidative stress (monobenzone (MBEH)-induced) trigger activation of the Unfolded Protein Response (UPR) in melanocytes (Manga 2010, Toosi 2012). Activation of the PERK arm of the UPR promotes reinstatement of homeostasis and stimulates cytoprotective pathways, such the Nrf2 antioxidant response (Cullinan 2004). If homeostasis cannot be restored, PERK-mediated expression of CHOP promotes apoptosis (Wang 1996). We have shown that melanocytes can evade apoptosis even under continual ER stress (Cheng 2013). In this study, we further explored a role for the PERK pathway in the melanocyte stress response and survival. **Methods:** We either inhibited PERK in human melanocytes using kinase inhibitor GSK2606414 or silenced PERK using an shRNA approach. Melanocytes were then exposed to MBEH and cell viability was determined. PERK pathway activity was monitored by Western blot analysis of PERK targets Nrf2 and eIF2α. **Results:** GSK2606414-mediated PERK kinase inhibition sensitized melanocytes to sub-toxic MBEH doses and reduced Nrf2-regulated activation of antioxidant responses as determined by expression of HMOX1. shRNA-PERK silencing resulted in an 88% decrease in melanocyte viability in short term cultures (3 days). Continued culture of the PERK-silenced melanocytes (14 days) resulted in improved viability (40% decrease). To further investigate PERK activity, we monitored a second PERK target, eIF2α. To promote a return to homeostasis in stressed cells, PERK phosphorylates eIF2α leading to repression of global translation. eIF2α is not typically phosphorylated at baseline in most cells; however a fraction of eIF2α was phosphorylated in cultured melanocytes suggesting that low level PERK activation is required to maintain melanocyte viability. Surprisingly, phospho-eIF2α levels were found to be elevated in 14-day cultures of PERK-silenced melanocytes suggesting that melanocytes adapt by activating an alternative kinase to phosphorylate eIF2α. **Conclusion:** Previous studies demonstrated that prolonged low-level eIF2α phosphorylation protects against ER stress (Walter and Ron 2011). Melanocytes displayed low-level basal PERK activation and inhibition of PERK resulted in loss of this protection and enhanced cell death. Sustained PERK down-regulation promoted adaptation through activation of an alternative kinase to phosphorylate eIF2α. We are currently identifying the mechanism underlying this adaptation and evaluating whether adaptation also protects melanocytes from chemical stressors. These pathways may prove to be dysfunctional in disorders where melanocyte viability is compromised, such as vitiligo, and/or play a role in extreme chemoresistance typical of transformed melanocytes in melanoma.

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Mixed-mode oscillations and population bursting in the pre-Bötzinger complex

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This study focuses on computational and theoretical investigations of neuronal activity in the pre-Bötzinger complex (pre-BötC), a medullary region generating the inspiratory phase of respiration. Progressive increase of neuronal excitability in medullary slices containing the pre-BötC produces mixed-mode oscillations (MMOs) characterized by large amplitude (LA) population bursts alternating with a series of small amplitude (SA) bursts. We demonstrate that these MMOs emerge within a heterogeneous neural network because of progressive neuronal recruitment and synchronization. The features of the MMO pattern depend on the distribution of neuronal excitability, the sparsity and weight of network interconnections, and the cellular properties underlying endogenous bursting. The latter should provide a reduction of spiking frequency within neuronal bursts with increasing burst frequency and a dependence of the after-burst recovery period on burst amplitude. Our study provides important insights into the generation of rhythmic activity by neuron populations representing central pattern generators in vertebrates.

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Targeting aerobactin biosynthesis in hypervirulent Klebsiella pneumoniae

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Since it was initially described in the mid-1980s in the Asian Pacific Rim, a hypervirulent strain of Klebsiella pneumoniae (hvKP) has since disseminated throughout the globe. In contrast to classical strains of Klebsiella pneumoniae (cKP), hvKP is able to cause serious life-threatening infections in previously healthy individuals in the community. There is fear amongst professionals in the medical community that convergence of this hypervirulent pathotype with the increasingly problematic strains of drug-resistant KP could lead to the evolution of a true "superbug" that would likely require novel therapeutics to combat. Recent work has demonstrated that the enhanced virulence of hvKP is, above all, mediated by overproduction of the siderophore aerobactin. Siderophores are small molecule iron-chelators that allow bacteria to acquire sufficient quantities of this vital nutrient in the severely iron-limited host environment. We hypothesize that inhibition of aerobactin biosynthesis could be a viable therapeutic target that could yield a novel "anti-virulence" pharmaceutical treatment for infections with hvKP and other pathogenic bacteria that rely on this siderophore. Toward targeting aerobactin biosynthesis for chemical inhibition, we aim to lay the ground work by structurally and functionally characterizing the four enzymes required to synthesize aerobactin (IucA-D), with specific emphasis on the synthetases IucA and IucC. Herein, we present a structural analysis of the synthetase IucA using X-ray crystallography and small-angle X-ray scattering (SAXS). In addition, we report the kinetic characterization of IucA, the development of an assay to be used in high-throughput screening for small-molecule inhibitors of IucA, and our initial screening results.

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Depletion of fat regulatory T cells prevents age-associated insulin resistance

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Age-associated insulin resistance (IR) and obesity-associated IR are two physiologically distinct forms of adult onset diabetes. While macrophage-driven inflammation is a core driver of obesity-associated IR, the underlying mechanisms of the obesity-independent yet highly prevalent age-associated IR are largely unexplored. Comparative adipo-immune profiling (AIP) reveals that fat-resident regulatory T cells, termed fTregs, accumulate in adipose tissue as a function of age, but not obesity. Supporting the existence of two distinct mechanisms underlying IR, mice deficient in fTregs are protected against age-associated IR, yet remain susceptible to obesity-associated IR and metabolic disease. In addition, selective depletion of fTregs via anti-ST2 antibody treatment increases adipose tissue insulin sensitivity. These findings establish that distinct immune cell populations within adipose tissue underlie aging- and obesity-associated IR and implicate fTregs as adipo-immune drivers and potential therapeutic targets in the treatment of age-associated IR.

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Targeting of the Deubiquitinase STAMBP with a Novel Small Molecule Inhibitor Inhibits NALP[7] Inflammasome Activity

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Introduction: Innate immune signaling by the inflammasome contributes to the pathogenesis of the acute respiratory distress syndrome (ARDS). Inflammasomes are multi-protein complexes that critically regulate the early immune response by facilitating the maturation and release of the potent pro-inflammatory cytokines IL-1b and IL-18. Increased circulating IL-1b and IL-18 are associated with increased mortality in ARDS. **Rationale:** NACHT, LRR, and PYD domains-containing protein 7 (NALP7) is one inflammasome constituent, but little is known about its cellular handling and regulation. Elucidation of the mechanisms of NALP7 handling may identify novel therapeutic targets to inhibit aberrant inflammasome signaling in ARDS and other inflammatory disorders. **Methods:** Cell culture and treatment, recombinant genetics, immunofluorescence microscopy, ELISA, Western blotting, co-immunoprecipitation, qPCR, in silico modeling and drug design, and *in vitro* DUB and caspase activity assays. **Results:** Upon exposure to Toll-like receptor (TLR) ligands, NALP7 forms an inflammasome with ASC and caspase-1 as shown by immunofluorescence microscopy, and knockdown of NALP7 inhibits normal release of IL-1b. Similar to NALP3, TLR ligands increase the protein abundance of NALP7 in a time-dependent fashion. NALP7 is modified by K63-linked ubiquitination, which tags NALP7 for degradation at the lysosome. NALP7 is recruited to the endo-lysosomal pathway by signal transducing adaptor molecule (STAM), a component of the Endosomal Sorting Complexes Required for Transport (ESCRT) pathway. Knockdown of STAM prevents NALP7 from entering the endosomal pathway and preserves NALP7 protein. NALP7 is stabilized by the deubiquitinase (DUB) activity of STAM-binding protein (STAMBP), an endosome-associated DUB. Knockdown of STAMBP abrogates the TLR agonist-induced increase of NALP7 protein abundance and inhibits secretion of IL-1b. Next, we developed a novel small molecule inhibitor of STAMBP DUB activity. Treatment of cells with this compound inhibits the TLR agonist-induced increase in NALP7 protein abundance and inhibits secretion of IL-1b. **Conclusions:** These findings suggest that NALP7 is pro-inflammatory, capable of inflammasome formation, and important for normal cytokine release. We demonstrate that NALP7 interacts with the ESCRT

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protein STAM for recruitment to the endosome and subsequent trafficking to the lysosome for degradation. The DUB activity of STAMBP rescues NALP7 from degradation when cells are exposed to TLR agonists. Our small molecule inhibitor establishes STAMBP as a potential therapeutic target to reduce the effects of injurious inflammasome-driven pro-inflammatory stress.

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Extending Pediatric Developmental Screening Through Faith-Based Partnerships

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Purpose: Medical home access in Genesee County/Flint, Michigan is limited by socioeconomic factors including poverty, health illiteracy, transportation, and lack of social support. Additionally, Flint is in the midst of a public health emergency with lead leaching into the water supply, increasing the risk of lasting neurodevelopmental effects. Community-based strategies to assist in early identification of those at risk of developmental delay, coupled with referral to the medical home for formal evaluation, are likely to be of tremendous benefit. As an integral part of the social fabric of the community, faith-based organizations in particular carry a heavy load in ensuring the community's welfare. We aimed to (1) empower the faith-based community to screen for pediatric developmental delay, (2) assess the feasibility of arming the faith-based community with skills to detect developmental delay, and (3) assist in establishing health insurance and medical homes for the community. **Methods:** Following IRB approval, the Ages and Stages Questionnaire (ASQ-3™) developmental screenings were administered by trained volunteers at five Genesee County churches to children aged 1 month to 5.5 years over a 5 month period. Parent/guardian surveys regarding satisfaction of the screening process were simultaneously administered. Quantitative and qualitative analyses were conducted on parent/guardian surveys and the completed ASQ-3s. **Evidence Base:** Faith-based organizations are community cornerstones: hosting social activities, disseminating information, organizing the community, and providing social services. Church-led interventions and screening initiatives have historically been successful in improving the health of underserved and hard-to-reach populations. **Results:** We aim to train 5 churches and analyze 200 parent-completed ASQ-3s and related surveys by the end of February 2016. Currently, volunteers at all 5 churches have been trained and survey/screener administration is underway. Data from 16 completed surveys revealed 50% of children have had previous developmental screenings, 44% of which were within the past year. 50% of children screened scored close to or below the cutoff suggesting risk for developmental delay in one or more categories, with the highest portion (31%), in problem solving. All parents/guardian participants thus far reported very high satisfaction with the ASQ-3 screening. **Conclusion:** Preliminary results suggest developmental screening by faith-based communities is positively received and is useful in screening and identifying risk of delay in children who would otherwise be missed. Additional findings, interpretations, and recommendations for others considering a similar approach are forthcoming, pending completion of our data collection.

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NELF-mediated RNA Pol II Pausing affects the TGFb Pathway

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Pausing of RNA polymerase II (RNA Pol II) near gene promoters is a key regulatory step that was originally identified in Drosophila (Rougvie and Lis 1988); here we investigated promoter proximal pausing in human cells. Pausing occurs when RNA Pol II is held in a transcriptionally active state 20-60 nucleotides downstream of the transcription start site through its interaction with two pausing factors, negative elongation factor (NELF) and DRB sensitivity-inducing factor (DSIF). RNA Pol II pausing has been shown to regulate the expression of MYC, FOS and JUNB (Krumm, A. et al. 1992, Plet, A. et al. 1995, Aida, M. et al. 2006). The goal of this study is to broaden our understanding of the role of RNA Pol II pausing on human gene expression. We identified over 12,700 genes that are enriched for RNA Pol II in the promoter region as compared to the gene body via PRO-Seq (Kwak et al., 2013). We found that NELF plays a key role in maintaining RNA Pol II promoter-proximal pausing, since silencing of NELF resulted in loss of pausing in more than 6,600 genes and a change in expression of more 1,000 genes. These genes include members of the TGFb signaling pathway such as BMP1, TGFB1, TGFBR1, SMAD2, SMAD3, SMAD6, SNAI1, CDKN2B and COL21A1. Our findings show for the first time that NELF-mediated RNA Pol II pausing regulates the expression of genes in the TGFb signaling pathway. TGFb signaling mediates a diverse set of cellular processes including inflammatory response and tumorigenesis. Our results identified a fine-tuning step that regulates gene expression in these cellular processes thus providing a novel target mechanism for therapies of immune diseases and cancer.

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The Role of Aspartate Aminotransferase in Peripheral Inflammation and Nociception

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Painful inflammation leads to a local increase in neurotransmitter Glutamate in the rat peripheral terminal (skin) and sciatic nerve. The primary sensory neurons of the dorsal root ganglia (DRG) are glutamatergic utilizing glutaminase (GLS) in the glutamine cycle as a source for glutamate production, but aspartate aminotransferase (AST; GOT1) also plays a key role by transamination of aspartate and 2-oxoglutarate to produce

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glutamate and oxaloacetate. We previously demonstrated that AST, GLS, and glutamate are elevated in the rat DRG and skin during the adjuvant-induced arthritis (AIA) model. Inhibition of GLS in the skin during AIA provides long-lasting analgesia. Since AST contributes to glutamate synthesis in peripheral nerve terminals, we hypothesized that the peripheral inhibition of AST using Aminooxyacetic acid (AOAA) would lead to analgesia during AIA. For the AIA model, peripheral inflammation was induced by intraplantar injection of Complete Freund's adjuvant in the right hind paw. After 4 days of inflammation, rats where treated with subcutaneous administration of AOAA. After AOAA administration, exaggerated thermal and mechanical responses in AIA rats were attenuated by 30 min and lasted for up to 5 hours. The current results further indicate that AST is a major contributor of glutamate synthesis in nociceptive peripheral nerve terminals. AST inhibition appears to be a viable target for producing long lasting local analgesia by decreasing glutamate production and release from peripheral primary afferents. NIH Grant AR047410

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Computational Modeling of Antidepressant Drug Effects

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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. In order to elucidate reasons behind the low efficacy rates of SSRIs and ultimately generate hypotheses to increase the effectiveness of pharmacological intervention, 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 monosynaptically. 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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A neurologic syndrome related to homozygous MAG gene mutation affecting sialic acid binding in myelination-associated glycoprotein

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The MAG gene encodes the myelin-associated glycoprotein, an abundant sialic acid-binding protein that localizes to central and peripheral myelin sheaths, and is involved in axon-glial interactions and myelination during nerve regeneration. In fact, it is a potent inhibitor of neurite outgrowth from neurons both *in vitro* and *in vivo*. Given its abundance and the fact that anti-MAG antibodies are associated with a prominent peripheral neuropathy, it is surprising that it has not been linked to Mendelian disorders until very recently. In fact, several members of a consanguineous family with MRI findings consistent with a demyelinating leukodystrophy and a clinical syndrome reminiscent of Pelizaeus-Merzbacher disease were recently reported with a homozygous missense c.399C>G; p.S133R mutation in MAG; this mutation caused the protein to be retained in the ER and degraded. Here we report a non-consanguineous family with a pair of brothers afflicted with a syndrome characterized by cognitive impairment, neuropathy, ataxia, nystagmus, and gait disorder. Exome sequencing revealed a homozygous missense mutation, p.Arg118His, in MAG. This arginine residue in Immunoglobulin domain 1 of the MAG protein is known to be critical for sialic acid binding, providing a clear mechanistic basis for the disorder. Interestingly, this region is not involved in MAG-related neurite inhibition, which involves Immunoglobulin domain 5. Thus, this mutation is expected to impart a distinct abnormality to the sialic acid binding ability of MAG. Future studies should reveal whether patients with mutations in distinct MAG regions have different clinical syndromes.

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Associations between Inflammation and Immunosenescence in HIV-associated Pulmonary Dysfunction

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Hypothesis: HIV infection is characterized by persistent immune activation and inflammation, which results in accelerated presentation of non-AIDS co-morbidities such as chronic obstructive pulmonary disease (COPD). Leukocyte telomere attrition is a characteristic immunosenescence marker in HIV infection. Limited studies have shown correlations between shorter telomere length and COPD in HIV-infected individuals; whether these associations differ in HIV-infected and HIV-uninfected populations with lung dysfunction has not been studied. We hypothesize that shorter telomere length and

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expression levels of circulating inflammatory cytokines are associated with pulmonary dysfunction and vary with HIV status. **Methods:** HIV-infected and HIV-uninfected men from the Multicenter AIDS Cohort Study were enrolled. Demographic and clinical data were obtained by chart abstraction or self-report. Peripheral blood mononuclear cells from each participant were measured for telomere length by quantitative real-time PCR. Plasma levels of associated inflammatory cytokines including interleukin (IL)-1ra, IL-4, IL-6, IL-13, and tumor necrosis factor (TNF)-α were measured by Luminex (Bio-Rad Laboratories Hercules, CA, USA). All participants performed standardized pulmonary function tests (PFTs). PFT values were dichotomized above and below accepted clinical cut-off points for airflow obstruction (post-bronchodilator forced expiratory volume in one second/forced vital capacity [FEV1/FVC] < or > 0.70) and moderate impairment in diffusing capacity for carbon monoxide (DLCO> or <0.6). Associations between telomere length and circulating inflammatory markers were made by Spearman's correlation. Comparisons between telomere length and dichotomized PFT values were made and adjusted for smoking and HIV status by logistic regression. **Results:** The cohort consisted of eighty men with average age of 52years. Thirty-two were HIV-infected, of which 81.5% were on ART, with a median CD4 cell count of 574 (172-1135). Overall, shortened telomere length was associated with airflow obstruction and diffusion impairment [mean telomere length post-bronchodilator FEV1/FVC above or below 0.7: 1.09 vs. 0.82 p=0.01 and DLCO above or below 0.6: 1.10 vs. 0.90 p=0.01], regardless of HIV and smoking status. Plasma levels of inflammatory cytokines were negatively correlated with telomere length [IL-1ra coeff =-0.36 p=0.00 IL4 coeff= -0.24, p=0.036; IL6 coeff= -0.24, p=0.029; IL13 coeff= -0.34, p=0.001; TNFα coeff= -0.34, p=0.012]. **Conclusions:** Telomere length was inversely correlated with airway obstruction and diffusing capacity in a cohort of HIV-infected and HIV-uninfected men. Higher levels of the circulating inflammatory markers IL-1ra, IL-4, IL-6, IL-13, and TNF-α were also associated with telomere attrition, suggesting an interaction between the peripheral inflammatory milieu and markers of aging. While no specific associations were found in HIV-infected persons, the small sample size was likely underpowered to detect such an association.

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Evidence of Fatty Acid Metabolic Defects and Right Ventricular Lipotoxicity in Human Pulmonary Arterial Hypertension

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Background: The mechanisms of right ventricular (RV) failure in pulmonary arterial hypertension (PAH) are poorly understood. Abnormalities in fatty acid (FA) metabolism have been described in experimental models, but systemic and myocardial FA metabolism have not been studied in human PAH. We hypothesized that FA defects are a feature of human PAH and contribute to altered RV metabolism. The importance of characterizing these pathways in humans is that existing therapies for insulin resistance favorably modify FA metabolism and therefore may offer a new strategy for targeting RV failure in patients with PAH. Μετηοδσ ανδ Ρεσυλτσ: We used human blood, RV tissue, and non-invasive imaging to characterize multiple steps in the FA metabolic pathway in PAH subjects and controls. Circulating free FAs ($p < 0.001$) and long-chain acylcarnitines ($p < 0.003$) were elevated in PAH patients versus controls after correcting for multiple comparisons. Human RV long chain FAs were increased and long chain acylcarnitines were reduced 100-fold in PAH versus controls. Using proton magnetic resonance spectroscopy to measure *in vivo* intramyocyte lipid in humans, we found myocardial triglyceride content was 7-fold higher in PAH subjects versus controls (1.4 ± 1.3 %TG vs. 0.22 ± 0.11 %TG, $p = 0.02$). Ceramides, pro-apoptotic mediators of lipotoxicity, were increased in PAH RVs versus controls ($p = 0.006$), suggesting that lipotxicity is a functional consequence of RV steatosis. Using an animal model of heritable PAH we demonstrated reduced fatty acid oxidation via failure of palmitoylcarnitine to stimulate oxygen consumption in the PAH RV ($p < 0.0003$). Χονχλυσιονσ: Abnormalities in fatty acid metabolism are detectable in the blood and myocardium in human PAH and are associated with RV lipotoxicity. Murine data suggests that lipotoxicity may arise from impaired fatty acid oxidation. This work highlights new pathways of potential therapeutic interest in human PAH and establishes a novel non-invasive imaging tool to study FA metabolism *in vivo*. Further studies are needed to determine the functional significance of FA defects and whether metabolic therapies can improve RV function in PAH.

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Aberrant expression of the neuregulin receptor ErbB4 promotes the pathogenesis of malignant peripheral nerve sheath tumors

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Neurofibromatosis type-1 (NF1) is an autosomal dominant tumor susceptibility disorder affecting approximately 1:3500 children. The hallmark of the disease is the development of dermal and plexiform neurofibromas, the latter of which are capable of malignant transformation. The resulting tumors, called malignant peripheral nerve sheath tumors (MPNSTs) are the most common malignancy affecting NF1 patients, but they carry an abysmal prognosis. Surgical resection represents the only effective means of treating MPNSTs, however complete resection is frequently impossible as MPNSTs aggressively invade adjacent tissues and metastasize. To date, no effective chemotherapeutic agents have been identified and further research is needed to understanding the signaling cascades mediating MPNST pathogenesis Prior studies in our laboratory have shown that

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dysregulated signaling by the growth factor neuregulin-1 (NRG1) promotes the pathogenesis of MPNSTs. Curiously, at least some MPNSTs coexpress erbB3, the sole NRG1 receptor found in non-neoplastic Schwann cells, and erbB4, a second NRG1 receptor with distinct functional characteristics. This led us to hypothesize that aberrant erbB4 expression occurs commonly in MPNSTs and that erbB4 promotes tumorigenesis via actions distinct from those regulated by erbB3. We found that erbB4 was expressed in 9/9 human MPNST lines and 27/30 (90%) surgically resected MPNSTs. ErbB4 was also uniformly expressed in MPNSTs arising in a genetically engineered mouse model of these tumors (P0-GGFb3;Trp53+/- mice). To assess ErbB4 function in MPNSTs, we generated P0-GGFb3;Trp53+/-;Erbb4flox/flox mice and ablated Erbb4 in MPNSTs derived from these animals. Compared to non-ablated controls, Erbb4-null MPNST cells demonstrated decreased proliferation and viability. Orthotopic allografts of Erbb4-null cells were also significantly smaller than control grafts and showed diminished proliferation and increased apoptosis. Erbb4 knockdown in human MPNST cells similarly inhibited DNA synthesis and cell viability. In contrast to our earlier demonstration that pan-erbB inhibitors decrease Ras activation, Erbb4 ablation did not disrupt Ras or MAPK activation. ErbB4 instead promoted the activation of the PI3K/Akt/mTOR, STAT3, STAT5, WNK-1 and phospholipase-Cγ signaling cascades. We conclude that aberrantly expressed erbB4 promotes the proliferation and survival of MPNSTs by activating key non-Ras dependent signaling cascades. ErbB4 and erbB4-regulated signaling cascades are thus potentially useful therapeutic targets in MPNSTs.

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Loss of intestinal epithelial autophagy leads to catastrophic susceptibility to acute T. gondii-mediated inflammation

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The protozoan parasite Toxoplasma gondii triggers severe small intestinal immunopathology characterized by intestinal inflammation, Paneth cell loss and transformation of the microbiota composition. Paneth cells are the predominant intestinal secretory epithelial cells which reside at the base of the crypt and release antimicrobial peptides to regulate the intestinal microbiota. T. gondii-triggered IFN-gamma is a major inducer of Paneth cell death. However, mechanisms of T. gondii-induced IFN-gamma-dependent Paneth cell death are currently unknown. We recently observed that under steady state conditions Paneth cells undergo active autophagy, which is both microbiota- and IFN-gamma-dependent. These findings directed us to investigate a role for autophagy in T. gondii –mediated Paneth cell loss. Mice lacking the critical autophagy gene Atg5 in the intestinal epithelium (E-ATG5KO) exhibited a catastrophic increase in susceptibility to intestinal inflammation, which was characterized by complete destruction of the intestinal crypts. To further investigate these findings we generated intestinal crypt organoids to study the mechanism underlying increased sensitivity to the cytokine mediated Paneth cell death in the absence of functional autophagy. Autophagy deficient organoids exhibited significantly increased sensitivity to the TNF-induced cell death. Organoid death was further exacerbated when TNF stimulation occurred in combination with IFN-gamma, indicating synergistic signaling from this inflammatory cytokine combination. Armed with this information, we returned to our *in vivo* model, which revealed higher expression levels of TNF receptor 2 (TNFR2) in E-ATG5KO mice than wild-type controls. This increase was further magnified during T. gondii infection in ATG5KO intestines. This indicates that E-ATG5KO mice are incapable of suppressing TNFR2 levels both at steady state and more critically during infection, leading to exaggerated sensitivity to cytokine-mediated immunopathology in the small intestine. Our results reveal that Paneth cell autophagy plays a highly protective role in the regulation of intestinal inflammation during acute T. gondii infection.

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Histone modifications predispose genome regions to breakage and translocation

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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. Using large-scale computational analysis, we identified altered chromatin features including specific histone marks at common leukemia and lymphoma breakpoints in hematopoietic stem cells. To probe the physiological relevance of these modifications, we 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 order to directly test the role of chromatin features in DNA breakage susceptibility, we 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 marks generally increase breakage susceptibility. Finally, we developed a high-throughput break-apart FISH (hiBA-FISH) assay to detect low frequency chromosome breakage and translocation events in lymphocytic cells expressing chromatin modifying enzymes. Experimental elevation of H3K4 methylation promotes chromosome breaks and specific translocations in response to genotoxic stress. Taken together, these experiments elucidate

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a novel role for histone modifications in the formation of nonrandom chromosomal breaks and the mechanisms that lead to translocations.

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FcγRIII signaling promotes IL-33-dependent monocyte migration to the lung extravascular space.

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IL-33 is implicated in type 2 inflammatory processes, including allergic asthma. Our group previously demonstrated that two Th2 stimuli, allergen-specific IgG immune complexes (ICs) and house dust mite extract (HDM), signal through FcRγ-associated receptors on antigen presenting cells to upregulate IL-33 in the lung and induce type 2 allergic airway inflammation. In this study, we investigated the role of IL-33 in regulating type 2 responses by comparing localization of allergen-positive monocytes in IL-33-deficient and IL-33-sufficient mice. We found that IL-33 is necessary for optimal monocyte accumulation in the lung extravascular space after allergen challenge and that treatment with pertussis toxin abolishes this migration. Our findings suggest that during allergic sensitization, activation of the FcRγ signaling pathway promotes monocyte migration into the lung extravascular space in a manner dependent on IL-33 and GPCR signaling, where monocytes may then contribute to type 2 allergic inflammation.

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Multiallelic Positions in the Human Genome: Challenges for Genetic Analyses

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As the amount of human genomic sequence available from personal genomes and exomes has increased, so too has the observation of genomic positions having two or more alternative alleles, so-called multiallelic sites. For portions of the haploid genome that are present in more than one copy, including segmental duplications, variation at such multisite variant positions becomes even more complex. To better understand such variants, we explored the frequency of multiallelic sites in large samples with whole exome sequencing. We find among our in-house sample sets of 14,443 individuals that 50,935 of 2,268,345 positions (2.24%) that were observed to be variant are actually multiallelic. Relatedly, 4.12% of all genotype calls occur at these multiallelic positions. Comparison with Illumina HumanExome BeadChip data from the same sample set reveals excellent concordance, suggesting the multiallelic variants we detected are indeed true variants rather than sequencing errors. Yet, despite the frequency of multiallelic variants, many analyses fail to directly address this class of variation. Likewise, a number of commonly used resources and tools in genomic research and diagnostics do not support multiallelic variants all together or require special modifications. As an example, we analyzed data from the NHLBI Exome Sequencing Project (ESP) Exome Variant Server, which does not support or contain any multiallelic SNVs. We identified 17 stop-gain/loss alleles that occur in >0.1% of individuals in our in-house sample sets yet are not included in ESP because they are masked by other alleles recorded at the same genomic position. An estimate of the upper bound suggests that 113,538 potential stopgain or loss alleles would be masked by other alleles in the ESP if they did occur. If databases commonly used for variant interpretation do not contain multiallelic variants, misinterpretation of variant frequencies can potentially occur. Likewise, failing to account for multiallelic variants in personal genomes sequenced clinically could theoretically lead to missed diagnoses. Thus, we encourage researchers and diagnosticians to determine if a specific use case requires consideration of sites that could potentially vary in more than one way. If so, we suggest accessing a resource that offers full compatibility.

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Follistatin-like Protein-1 Limits Inflammation in Acute Lung Injury

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Introduction: Follistatin-like protein 1 (FSTL-1) is a secreted protein that mediates inflammation in *in vitro* and *in vivo* models, including our prior studies using FSTL-1 Hypomorphic mice. FSTL-1 is produced in the lung and plays a critical role for normal lung development and repair following injury. The role of FSTL-1 in lung inflammation is unknown. We sought to evaluate the role of FSTL-1 in an LPS induced acute lung injury model. **Methods:** FSTL-1 Hypomorphic mice underwent oropharyngeal (o.p.) inoculation with PBS or 500ug/kg LPS (E. coli O111:B4). Similarly, C57Bl/6 (WT) mice were treated with either 200ug o.p. polyclonal IgG or αmFSTL-1 on Day -1 followed on Day 0 by o.p. LPS. At 4 hours post-LPS treatment, bronchoalveolar lavage fluid was collected, as was lung tissue for protein and RNA isolation. Lung FSTL-1 protein expression was assessed by Western blot densitometry. **Results:** In WT mice FSTL-1 protein increased following LPS treatment; compared to WT mice, FSTL-1 hypomorphic mice had decreased FSTL-1 protein with and without LPS stimulation. Following LPS treatment, FSTL-1 Hypomorphic mice had increased BALF neutrophils compared to WT mice and PBS-treated FSTL-1 Hypomorphs, which was associated with increased transcription of csf3, il6 and cxcl2 in whole lung. With or without LPS treatment, αFSTL-1 treated mice had increased BALF total cells and neutrophils compared to IgG treated controls. Without LPS treatment, αFSTL-1 treated mice had increased il17a, il6 and cxcl2 compared to IgG treated controls. **Conclusions:** Using parallel *in vivo* approaches

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to examine the role of FSTL-1 in LPS induced acute lung inflammation, our data show that FSTL-1 is induced following LPS administration. Further, attenuation of FSTL-1 results in increased neutrophilic presence in BALF, which is associated with increased cytokine and chemokine transcript abundance. This suggests that FSTL-1 may exert a homeostatic, anti-inflammatory role in the mammalian lung such that disruption of FSTL-1 may lead to lung inflammation. FSTL-1 may function to constrain the induction of pulmonary inflammatory processes and may be a potential avenue for further investigation.

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MYD88 and TICAM-1 mediate compensatory pro-leukemic signaling in acute monocytic leukemia

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Acute myeloid leukemia (AML) is an aggressive malignant neoplasm arising from hematopoietic stem cells and encompasses several clinically distinct subtypes. AML is most frequently treated with combination anthracycline and cytosine arabinoside chemotherapy. However, this approach is yields a five-year survival of 25% of all patients and 5-10% of elderly adults, who are most frequently afflicted with AML. Accordingly, an urgent need persists for characterization of novel oncogenic pathways in AML that may be exploited for therapeutic benefit. Acute monocytic leukemia (AMoL) is a subtype of AML characterized by an excess of monocytes and their progenitors that corresponds to M4/M5 FAB subtypes. We have shown in AMoL primary patient samples that myeloid differentiation response gene 88 (MYD88), Toll-like receptor 1 (TLR1), TLR2, TL4, TLR5, Toll-like receptor adaptor molecule 1 (TICAM-1) and other TLR-associated genes are overexpressed in these AML subtypes. Further, we developed a murine MLL-AF9 leukemia cell line (LCs) with conditional Myd88 deletion (Myd88fl/fl). Upon deletion of Myd88, we observed reduced colony formation and proliferation as well as delayed leukemia development. Over extended suspension culture, we observed a recovery and normalization of colony forming ability of LCs following conditional deletion of Myd88, suggesting a partial compensatory survival response. Accordingly, development of Myd88-/- LCs from a mouse with germline Myd88-/- genotype more closely resembled wild-type LCs developed in parallel. Of note, these Myd88-/- LCs expressed high levels of Ticam-1, an alternative Tlr adaptor. Likewise, we have developed MYD88-null human cell lines using CRISPR/Cas9 genome editing. We are currently investigating the hypothesis that TICAM-1 mediated signaling partially compensates for MYD88 loss. With the expanding application of IRAK inhibitors that effectively inhibit MYD88-dependent signaling, TICAM-1 overexpression and signaling might represent a mode of resistance to these treatments, making it a potentially clinically significant phenomenon.

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P-Glycoprotein Activity Quantification in Pediatric Patients with Acute Lymphoblastic Leukemia

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Introduction: P-glycoprotein (P-gp) is an ABC protein that mediates the ATP-dependent efflux of hydrophobic toxins. Physiologically P-gp excretes endogenous metabolites and other xenotoxic substrates into the urine, bile and feces. P-gp is a Multidrug Resistance (MDR) pump, which exports chemotherapeutic agents from target cells to the extracellular space. In some studies, higher P-gp expression has been described in relapsed more than initial diagnosis of leukemia and was related to increased risk of relapse, because efflux of chemotherapeutic drugs reduced the intracellular levels, producing an increase demand of higher doses to produce an equivalent effect as before the P-gp overexpression. Recent studies have shown that the expression of certain gene products mediate the development of resistance to chemotherapeutic agents. The best characterized of these genes is the multidrug resistance gene MDR-1. P-gp overexpression studies in leukemia during childhood, have been showed a large variability between the methodology and the results in each study, they conclude that the most adequate procedure must quantify or get a directly measure gene expression. **Method:** This study is planning to study the expression of P-glycoprotein in patients with acute leukemia and the effect in cell incubated with Daunorrubicine. The study is carrying out in 100 patients with acute lymphocytic leukemia. Flow cytometric analysis of P-gp surface expression is performed. **Results:** Final results are expect in February 2016.

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External Validation of the NEXUS Chest Decision Instruments for Selective Chest Imaging in Blunt Trauma

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The NEXUS Chest decision instrument (DI) was derived to reduce unnecessary chest imaging of blunt trauma patients over 14y/o, by allowing clinicians to identify and avoid scanning patients who have low risk of significant intrathoracic injury that would be visible on chest radiography. This DI consisted of chest pain, distracting injury, chest wall tenderness, intoxication, age >60years, rapid deceleration, and altered alertness/mental status. It was internally validated in an observational prospective study of 9905 blunt trauma patients, and demonstrated a sensitivity (SN) of 98.8% and a negative predictive value (NPV) of 98.5%. In order to broaden the range of useful DIs available, the NEXUS group also derived two similar but more specifically targeted instruments, the CHEST CT-All DI (abnormal chest radiograph, rapid deceleration, distracting injury, chest wall/sternal/thoracic spine/scapular tenderness) to maximize sensitivity

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for all injuries and the CHEST CT-Major DI (same criteria without rapid deceleration) to maximize sensitivity for only major thoracic injuries. While all of these DIs have been internally validated, none of them have been externally validated. In order to test the performance of the NEXUS Chest, CHEST CT-All, and CT-Major DIs at our institution, we retrospectively reviewed 2700 cases of traumatically injured patients presenting to our urban academic ED, excluding duplicate charts, transfers, incomplete evaluations, those without blunt trauma or possible thoracic injuries. On initial analysis, 1166 patients met inclusion criteria, and the three DIs were applied. Applying the NEXUS Chest DI to blunt trauma patients identified 595/1166 (51%) patients who were deemed very low risk. Of these patients, 117 had received an unnecessary chest radiograph according to the DI, of which no significant injuries were found. Applying the CT-All DI, of the 180/1166 (15%) patients who met criteria, 3 received an unnecessary CT-chest, of which no significant injuries were found. When applying the CT-Major DI, of the 179/1166 who met criteria, 3 received an unnecessary CT-chest without any significant injuries being found. In contrast, these 3 DIs correctly categorized 28 patients with injuries as needing imaging studies, including 9 patients with major thoracic injuries. Our study supports the use of the NEXUS Chest, CHEST CT-All, and CHEST CT-Major DIs in an urban academic emergency department to identify patients at very low risk of thoracic injury who do not need further imaging after blunt trauma.

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ALR protein, a critical protein in cardiac development, regulates cellular iron homeostasis and maturation of cytosolic Fe/S proteins by regulating mitochondrial transport of ATP-binding cassette (ABC)–B8

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Introduction: Disruption of Fe/S cluster maturation can lead to cellular iron accumulation and oxidative stress, as seen in the cardiomyopathy associated with Friedreich's ataxia. Augmenter of Liver Regeneration (ALR), a mitochondrial inter-membrane-space protein involved in mitochondrial protein import, is critical for cardiac development in zebrafish, and its deletion is associated with increased oxidative stress and cytosolic Fe/S cluster maturation defects. ABCB8 is one of only two mitochondrial membrane proteins known to regulate cytosolic Fe/S cluster maturation. We hypothesized that ALR is critical for cytosolic Fe/S cluster maturation and iron homeostasis by regulating mitochondrial import of ABCB8. **Results:** Downregulation of ALR *in vitro* resulted in reduced cytosolic Fe/S cluster-containing enzyme activities and increased cellular iron uptake. Since ALR has mitochondrial– and cytosolic-specific isoforms, we performed knockdown-rescue studies of each isoform, and demonstrated that only the mitochondrial ALR is needed for the maturation of cytosolic Fe/S clusters. Because Fe/S clusters are synthesized in the mitochondria, we then assessed whether ALR can alter the levels or activity of ABCB7 and ABCB8, the two mitochondrial proteins known to regulate the maturation of cytosolic Fe/S cluster proteins. Downregulation of ALR reduced the mitochondrial levels of ABCB8, while ABCB7 levels were not affected. We also identified defects in mitochondrial transport of ABCB8 as the mechanism for reduced mitochondrial ABCB8 levels with ALR knockdown. Finally, we demonstrated that ALR interacts with Mia40 (a protein required for the import of certain mitochondrial proteins) to regulate the transport of ABCB8 but not ABCB7 into mitochondria. **Conclusion**: Our results indicate that ALR and its interaction partner Mia40 are involved in the transport of ABCB8 into the mitochondria, which in turn regulates cytoplasmic Fe/S cluster maturation. These findings provide insights into cellular iron regulation, with implications in cardiovascular disease.

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Pathologic Comparison of Angiotensin Induced Superior Mesenteric Artery Aneurysms in Smooth Muscle Low-Density-Lipoprotein-Related-Receptor-1 Deficient Mice between Constitutive and Post-Maturity Deletion Models

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Objective: Recent genome wide association studies have shown a relationship between low-density-lipoprotein-receptor-related-protein-1 (LRP1) and presence of abdominal aortic aneurysms. LRP1 is a large, multifunctional receptor that is involved in a wide range of diverse processes: fetal development, cholesterol homeostasis, and protease clearance. The Daugherty Lab has previously demonstrated that constitutive deletion of smooth muscle cell LRP1 (smLRP1) in mice greatly exacerbates angiotensin (AngII) induced aneurysms. Unexpectedly, AngII infusion promoted a large luminal expansion of the superior mesenteric artery (SMA) and rupture at this location was associated with increased mortality in the sm-LRP1 mice. Due to the ubiquity of LRP1 expression during all stages of life, AngII induced aneurysm pathology in a constitutive smLRP1 deficient mouse may result from disruption of either LRP1 regulated vascular development or LRP1 mediated vascular homeostasis. The purpose of this study was to determine whether AngII-induced SMA aneurysms were different between mice with constitutive LRP1 deletion versus post-maturity induced LRP1 deletion. **Approach and Results:** Mice with a constitutive deletion of LRP1 were bred by crossing a Cre recombinase under control of smooth-muscle-specific promoter sm22 with LRP1 floxed mice (sm22-Cre x LRP1fl/fl). To determine whether LRP1 deficiency also exerted vascular effects when deleted in mature 8 week old mice, we bred mice expressing a tamoxifen-inducible Cre recombinase under the control of smooth-muscle-specific promoter sm22 with LRP1 floxed mice. The resulting SMA-ERT2-Cre x LRP1fl/fl mice were then injected with tamoxifen (1.5 mg/kg/day) for 5 days. Both groups were infused with AngII (1000ng/

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kg/min) for either 4 weeks or 12 weeks. Upon examination of SMA aneurysmal tissue, both groups showed similar luminal expansion, neointimal formation, elastin fragmentation, fibrotic tissue change, and macrophage infiltration. **Conclusion:** Because both models exhibit similar pathology, AngII induced aneurysmal changes in LRP1 deficient mice may be primarily the result of the loss of LRP1 mediated vascular homeostasis rather that LRP1 regulated vascular development. Thus, LRP1 plays an active and ongoing role in protection of vascular integrity post maturity. However, further studies are needed to determine if there is a vascular protective effect of restoring or upregulating LRP1 function in these models.

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Noncanonical NF-κB signaling in glioma is activated by TWEAK and promotes invasion

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High-grade gliomas are an invasive and deadly brain cancer. We have shown that noncanonical NF-κB/RelB signaling can drive invasion in the aggressive mesenchymal subtype of glioma. TNF-like weak inducer of apoptosis (TWEAK) can activate canonical/RelA and noncanonical/RelB NF-κB signaling, but its role in glioma invasion is unclear. Moreover, the relevance of expression of TWEAK and NF-κB-inducing Kinase (NIK) in patient gliomas is unknown. TWEAK-regulated NF-κB signaling and cell invasion were investigated in both established and primary high-grade glioma tumor lines using a three-dimensional (3-D) collagen invasion assay. NF-κB proteins regulating both glioma cell invasion and expression of Matrix Metalloproteinase 9 (MMP9) in response to TWEAK were evaluated using shRNA-mediated loss-of-function. NIK-promoted glioma growth *in vivo* was investigated using an orthotopic xenograft mouse model. Expression of TWEAK, its receptor, NIK, and a negative regulator of NIK (altogether termed the TWEAK Axis) in glioma tumors was evaluated using microarray data from the NCI-curated TCGA and REMBRANDT databases. TWEAK Axis expression was stratified by prognostic factor and glioma subtype as well as correlated with expression of both NF-κB genes and glioma subtype markers. Patient survival by TWEAK Axis gene expression was determined using Kaplan-Meier survival analysis. Protein levels of RelB correlate positively with glioma cell invasiveness. Loss of RelB attenuates invasion without altering RelA expression or phosphorylation. RelB promotes invasion independent of RelA. TWEAK selectively activates noncanonical NF-κB signaling through p100-p52 processing and nuclear accumulation of RelB and p52. TWEAK, but not TNFα, significantly increases NIK mRNA expression and sustains MMP9 mRNA expression. TWEAK-increased invasion is reversible by loss of NIK. NIK overexpression increases invasiveness *in vitro* and gliomagenesis *in vivo*. TWEAK, its receptor, and NIK are expressed primarily in mesenchymal gliomas and correlate negatively with survival. Our data establish a key role for NIK and TWEAK in noncanonical NF-κB signaling and MMP9-dependent invasion in glioma cells. Meta-analysis of patient glioma tumors indicates poorer prognosis with increased TWEAK Axis expression in the aggressive mesenchymal subtype. Together, these studies reveal the importance of TWEAK and noncanonical NF-κB signaling in glioma cell invasion and rationalize therapeutic targeting of TWEAK and NIK in glioma.

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Am I the Only One? An E-Learning Module for Student Mental Health

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In recent years, the mental health of health profession trainee has garnered prominent discussions in the media. Indeed, studies have suggested a high rate of depressive like symptoms in medical students as well as interns during the years of training. This is an urgent issue that must be addressed, not only because of the concerns for these students, but also for their training quality's effect on their future patient population. However, it is unclear what may be the best approach to destigmatize the subject and to encourage trainee participation in the necessary mental health self-care. In the era of online and technology assisted learning, this form of education may be an effective modality for the introduction of important concepts regarding trainee mental health. The utility of an E-Learning module allows anonymity, time flexibility, and may be incorporated into a flipped-classroom program, providing students with sufficient background to begin a meaningful conversation during classroom time. The current project was designed to educate participants on recognizing the signs and symptoms of anxiety, depression, and burnout in themselves and their colleagues. Contents of the learning module were designed after compilation of qualitative data from student anecdotal experiences. The module also incorporated reference lists of campus and community resources to help students through mental health challenge while in training. Topics included stress and anxiety management tools, such as mindful meditation, biofeedback, and counseling. Recommendations on preventive care and symptom management were also incorporated. To enhance interactive learning and user engagement, effective strategies taken from educational research literature were utilized, including animated conversation simulations and self-reflecting prompts dispersed throughout the module. The E-learning module will serve as a powerful foundation for campus-wide research into a variety of questions concerning student mental health, such as common problems experienced by trainees, perceptions on mental health care barriers, and interventions most appealing to students. It is hoped that by increasing discussion of health profession trainee mental health, the module will begin to destigmatize and normalize student experiences, and generate discussions among colleague to ensure that each and every student can receive the appropriate mental health care necessary for a successful training program.

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A (Perceived) Need for Speed: Prevalence and Perceptions of Medical and Non-Medical Prescription Stimulant Use Among Medical Students

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There are growing concerns among medical students and educators about inappropriate stimulant use by medical students. However, very few studies have evaluated the extent of both the medical use of prescription stimulants (prescribed stimulants, PS) for a diagnosis such as Attention Deficit Hyperactivity Disorder (ADHD), and the non-medical use of such drugs (non-prescribed stimulants, NPS), taken to enhance cognitive function in a medical school setting. The present study assesses the prevalence, perceptions, and beliefs about PS and NPS use at a southeastern medical school. All 781 matriculated students were asked to complete a 58-item, confidential, online questionnaire. The overall response rate was 59.8% (467/781), with current rates of stimulant medication use reported to be 16.5% for PS and 6.7% for NPS. This contrasted with the student body's perceived higher stimulant use rates at 25% and 20% respectively. The majority of students who used PS had self-reported diagnoses of ADHD (88.5%) and were first prescribed the drug during medical school (52.6%). NPS use typically started before medical school (72.2%). A third of the students using PS reported having been asked by peers to give/sell/trade their medication to others (34.5%), and 25.3% of all PS users actually made the transaction. More than a quarter of survey respondents (26.7%) reported being offered NPS by a classmate. Students without an ADHD diagnosis reported perceiving that students with a diagnosis of ADHD who use PS have an academic advantage over other students (53.1%). NPS users were more likely to disagree with the statements that NPS use is unethical (34.3% vs 10.4%), detrimental to the health of the user (34.3% vs 13.5%), and a serious problem among medical students (48.6% vs 14.5%) compared to non-users. We report higher rates of ADHD diagnoses and current PS and NPS use than previous studies of medical students. Additionally, the majority students with ADHD were diagnosed after beginning medical school. This finding is consistent with a previous study of ADHD among medical students, but in contrast to the typical diagnosis of ADHD, which occurs in childhood. Nearly one quarter of students reported using PS or NPS during medical school. Medical educators should look into strategies to reduce stimulant use that is not medically indicated among medical students and consider these factors when designing medical school curricula.

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Concomitant Targeting of c-Met and Epidermal Growth Factor Receptor Suppresses Pancreatic Cancer Cell Proliferation and Enhances of Gemcitabine's Effectiveness *in vivo*

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Pancreatic adenocarcinoma (PDAC) is the fourth leading cause of cancer-related deaths in the United States, and is expected to become the second leading cause by 2030. Due to its late stage at clinical presentation and its highly aggressive nature, PDAC patients have a poor prognosis with a median survival < 6 months. In addition to frequent major driver mutations (KRAS, SMAD4, TP53, and CDKN2A) and a plethora of other mutations, PDAC is associated with overexpression of tyrosine kinase receptors including EGFR and c-Met and their corresponding ligands. Bioinformatics analysis of the PDAC data set from The Cancer Genome Atlas (TCGA) revealed that ~14% of PDAC patients harbor upregulated MET gene expression. Furthermore, EGFR gene expression was upregulated in ~7% of patients, whereas EGFR protein expression was upregulated (~4%) or down-regulated in other cases. Importantly, patients that possessed alterations in both c-Met and EGFR had a statistically significant shorter survival than patients with EGFR alterations alone. Moreover, analysis of gene expression data from cancer cell line encyclopedia (CCLE) showed that ASPC-1 and PANC-1 pancreatic cancer cells (PCCs) expressed high levels of EGFR and Met. In this regard, we determined that the addition of cabozantinib (inhibits c-Met) or erlotinib (inhibits EGFR) decreased PCC proliferation and increased their sensitivity to gemcitabine as determined by MTT proliferation assay. Similar results were observed when PCCs were grown in a 3-dimesional culture system, in an orthotopic model, and a genetically engineered mouse model of PDAC. Therefore, targeting both receptors may enhance gemcitabine chemosensitivity and we suggest that this triple therapy should be tested in clinical trials.

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Airway Therapeutic Discovery Using a Porcine Model of Cystic Fibrosis

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Abnormal airway smooth muscle function is thought to contribute to airway hyper-reactivity. In cystic fibrosis (CF), airway hyper-responsiveness has been frequently reported and suggests that an altered airway smooth muscle (ASM) physiology may contribute to CF airway disease. The newborn CFTR-/- pig displays ASM abnormalities prior to the onset of inflammation or infection including increased basal tone, increased bronchodilator response, and decreased calcium reuptake. The goals of this study were to: 1) identify whole genome transcriptional changes in porcine CF ASM prior to

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airway inflammation and infection, 2) investigate whether specific pathways are associated with CF airway reactivity, and 3) determine if the CF pig transcriptome can be used to identify potential ASM therapeutics for CF and other airway narrowing diseases. Total RNA sequencing of newborn non-CF and CF ASM revealed differential and significant changes in muscle contraction related genes, ontologies, and pathways. We then used connectivity mapping to match small molecules that cause transcriptional signatures opposite of CF ASM. We hypothesized that by scanning for compounds that illicit a negative signature, we would enrich for therapeutic drugs in CF airway reactivity and other airway narrowing diseases. We found 90 compounds that were significantly enriched ($p<0.05$) that represent potential compounds that alleviate airway contractility and encompass many known modulators of airway hyper-responsiveness such as PI3K inhibitors, PDE inhibitors, prostaglandins, and beta-2 agonists. In addition to these known modulators of ASM function, several novel compounds were identified, with one being a kinase inhibitor. Wild-type precision cut lung slices pretreated with the kinase inhibitor demonstrated decreased airway narrowing in response to cholinergic stimulation compared to DMSO controls. Additionally, pretreatment with the compound in isolated wild-type smooth muscle strips diminished force production and decreased myosin light chain phosphorylation. These studies show that loss of CFTR in ASM causes whole genome-wide transcriptional changes, including expected alterations in muscle contraction transcripts. With connectivity mapping, we were able to identify several compounds known to modulate airway smooth muscle function and numerous novel compounds. These results suggest that direct modulation of CFTR may be beneficial in the treatment of the asthma-like phenotype observed in CF. Moreover, by recognizing the newborn CF pig as a model of airway smooth muscle hyper-reactivity absent inflammation, we can enhance our molecular understanding of airway smooth muscle biology and pathophysiology in addition to discovering novel therapeutics for airflow limitation. Supported by American Asthma Foundation and Cystic Fibrosis Foundation.

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The role of HIV-1 Tat protein in bystander CD4 T cell pyroptosis versus apoptosis

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HIV/AIDS currently afflicts over 1.1 million people in the US, and the CDC projects that 50,000 new cases will emerge this year. Despite pharmacologic advances in treating HIV and delaying progression to AIDS, anti-retroviral therapy (ART) remains inadequate for this significant pathogen. 13,000 people in the US and 1.5 million people worldwide die from AIDS each year. Efforts to improve therapy have been hindered by a 30-year-long dearth of understanding of the mechanism by which HIV induces death of CD4 T cells, which is the population it selectively depletes to cause AIDS. Paradoxically, the majority of cells that die during HIV-1 infection are not productively infected themselves; they are known as bystander T cells. Therefore, understanding the mechanism by which HIV mediates T cell death in the bystander population is critical to identifying new potential therapeutic targets for preventing T cell death and immunodeficiency. Recent work by others suggests that pyroptosis, an inflammatory form of programmed cell death that culminates in cell lysis and release of inflammatory cytokines, may mediate bystander cell killing. However, previous work by other groups has suggested that apoptosis mediates bystander killing. We sought to clarify the relative contributions of pyroptosis versus apoptosis in HIV-1-mediated bystander killing, hypothesizing that the majority of bystander cells die by pyroptosis. We infected Jurkat T cells and peripheral blood lymphocytes (PBLs) from donors with the HIV-1 isolate NL4-3 for 4 days and measured p24 Gag expression as a marker of productively infected cells, as well as activated caspase-1, a marker for pyroptosis, and activated caspase-3,7, a marker for apoptosis, by FACS analysis. We found that most of the caspase-1 and caspase-3 activation we observed occurred in the p24-negative ('bystander') population. Moreover, in both Jurkat T cells and PBLs, more caspase-1 activation occurred in the bystander population than did caspase-3 activation. A candidate mediator of bystander killing is the HIV protein transactivator of transcription (Tat). Tat is known to be secreted by infected cells and readily taken up by bystander cells, and is also recognized to increase transcription of a number of cellular genes. We hypothesize that Tat positively contributes to HIV-mediated bystander killing by pyroptosis through activation of specific cellular genes. We have identified several candidate cellular genes that may be involved in bystander pyroptosis and are currently evaluating their roles. From these studies we expect to clarify the role of Tat in HIV-1-mediated bystander T cell death, potentially illuminating new targets to prevent T cell depletion and AIDS progression.

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Influenza A viruses with reductions in both polymerase error rate and viral pathogenicity may serve as novel live attenuated vaccine platforms

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Influenza A virus infects millions of persons and is responsible for over 250,000 deaths annually. Two main forms of vaccination are utilized as front line defense against this virus: inactivated and live. The inactivated vaccine has a well-characterized mechanism of action but poor efficacy in the young. LAIV on the other hand is superior to the inactivated vaccine in children under 7, but an inadequate safety profile prevents universal use in the pediatric population. Utilizing the newly published crystal structure of the viral polymerase we have developed novel vaccine candidates through decreasing the error rate of influenza A viruses. This was accomplished through mutations affecting the size and charge of the putative nucleotide entry channel. These viruses possess decreased error rates *in vitro* and are attenuated in a murine model of infection, yet are still immunogenic.

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Claudin-2 leads to defective proximal tubule calcium reabsorption, hypercalciuria, and nephrocalcinosis

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Kidney stones have persisted with high prevalence and cost to humanity throughout recorded history. The most common finding in recurrent kidney stone formers is an elevated urine calcium without any overt systemic cause, often referred to as idiopathic hypercalciuria (IH). One potential cause of IH is reduced reabsorption of calcium in the kidney, where the proximal tubule (PT) reabsorbs the majority of calcium in a process that is widely believed to be passive and paracellular. Indeed, recent studies using lithium clearance as a measure of PT reabsorption have found that decreased PT Ca2+ reabsorption is common in kidney stone formers. The permeability of the paracellular pathway is largely governed by claudins, a family of proteins found at the tight junction of epithelial cells. In the PT, there is high expression of the cation-selective claudin-2. We hypothesize that claudin-2 mediates PT calcium reabsorption, loss of which leads to nephrocalcinosis. We provide evidence that loss of claudin-2 results in increased calcium excretion in hemizygous male mice (Cldn2-/y) compared with their wild type (Cldn2+/y) littermates (FeCa: Cldn2+/y 0.058±0.012 n=9 vs. Cldn2-/y 0.140±0.028 n=7). Chronic hydrochlorothiazide (HCTZ) administration has been shown to decrease urine calcium by increasing reabsorption of calcium in the PT, so we tested the hypothesis that Cldn2-/y are defective in PT calcium reabsorption by treating mice with HCTZ for 6 days (25 mg/kg/day). Preliminary studies suggest that Cldn2-/y have a reduced response to HCTZ (difference UCa/UCr post- HCTZ, Cldn2-/y -0.248±0.077 n=5 vs. Cldn2-/y -0.050±0.072 n=5). Using microCT and histological analyses, we also present evidence that Cldn2-KO mice develop papillary nephrocalcinosis. In 1937, Randall first observed and described the initiating step in human idiopathic kidney stone disease as hydroxylapatite plaque formation, so called Randall's plaques. Randall's theory has since been elaborated upon to demonstrate that these plaques form on the basement membrane of thin limbs of the Loops of Henle (tLOH). Thus, we are investigating the potential for Cldn2-/y mice as an exciting new model for the study of human kidney stone disease.

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The F-box protein cyclin F coordinates the levels of DNA replication-dependent histones with DNA synthesis

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The F-box protein cyclin F is the substrate recognition subunit of an SCF (Skp1, Cul1, F-box protein) ubiquitin ligase complex. We have previously established a role for cyclin F in maintaining both chromosome and genome integrity by inhibiting over-duplication of centrosomes and regulating the balance of the cellular dNTP pool. Here we identify a novel function of cyclin F in targeting stem-loop binding protein (SLBP) for proteasome-mediated degradation during the S- to G2-phase transition of the cell cycle. SLBP is necessary for the processing, translation, and degradation of replication-dependent histone (i.e., canonical histones) mRNAs during S-phase. Knockdown of cyclin F leads to stable SLBP throughout G2-phase of the cell cycle . Concluding experiments on this project are focusing on the cell's capability to maintain genome integrity and repair DNA damage in the presence of abnormally high levels of SLBP. Our work will both further define cyclin F's role in maintaining genome stability and identify an important function for this F-box protein in the regulation of canonical histone levels.

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Ceramide accumulation in mitochondria: a novel therapeutic strategy for Acute Myeloid Leukemia via inducing lethal mitophagy

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Mutations in FLT3 receptor tyrosine kinase are common targets in Acute Myeloid Leukemia (AML); however, FLT3 targeted therapy shows limited success due to development of resistance. Ceramide, a bioactive sphingolipid, is synthesized de novo by Ceramide Synthases (CerS) and mediates cancer cell death in response to various chemotherapeutic agents. This study investigates the biological role of ceramide lipid in the response of AML to FLT3 targeted therapy and aims at finding mechanism-based alternative therapeutic strategies to overcome resistance to FLT3 inhibitors. We found that AML cell lines and patient samples expressing FLT3 have suppressed CerS1 expression and lower levels of its product C18-ceramide compared with FLT3 negative AML cells. Silenced FLT3 expression or its pharmacological inhibition increased CerS1 and C18-ceramide levels while FLT3 overexpression suppressed them. The increase in C18-ceramide after FLT3 inhibition is required for cell death as silencing CerS1 expression or inhibiting its enzymatic activity protected from FLT3 inhibitors-induced cell death *in vitro* and *in vivo*. Mechanistically, FLT3 inhibition resulted in CerS1 translocation from cytosol to mitochondria resulting in generation and mitochondrial accumulation of C18-ceramide. The mitochondrial C18-ceramide then binds directly to LC3B-II to recruit autophagosomal membranes to mitochondria for the

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execution of lethal mitophagy and degradation of mitochondria. We also show that this process is regulated upstream by early Drp1 activation and p-Drp1 S637 de-phosphorylation, whereby silencing Drp1 expression or preventing its S637 dephosphorylation blocked the translocation of CerS1 to mitochondria, prevented ceramide mitochondrial accumulation, halted the events of lethal mitophagy, and protected from FLT3 inhibitors-induced cell death. Due to the importance of ceramide accumulation in mitochondria for AML cells to respond to FLT3 inhibition, we proposed a synthetic lipid compound, LCL-461, composed of C18-ceramide conjugated to a pyridinium ring in the sphingosine backbone. Mass spectrometry proved that LCL-461 accumulates selectively in mitochondrial fractions of AML cells due to the positively charged conjugated pyridinium ring. LCL-461 was effective in inducing cell death in several AML cell lines of different FLT3 mutation statuses and resistance profiles as well as in patient samples and *in vivo* xenografts, with minimal cytotoxicity effects on normal human bone marrow cells. LCL-461 induced cell death via the same LC3B dependent lethal mitophagy mechanism detected following FLT3 inhibition. This highlights the potential of LCL-461 as an agent that can bypass FLT3 signaling by accumulating in mitochondria to induce lethal mitophagy and AML cell death regardless of whether patients are sensitive or resistant to FLT3 targeted therapy.

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New Equations for Magnetization Components of Flowing Blood and Static Tissue Spins Under NMR/MRI Excitation

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This paper describes thoroughly the need and the method of derivation of the first of its kind two uncoupled differential equations describing the relations of single component My and Mz of magnetization (rotating frame) of flowing blood spins under NMR/MRI excitation with parameters of clinical importance such as blood flow velocity, relaxation times T1 and T2, and rf B1(x,t) field etc.. Corresponding equations for static tissue are also derived. These new equations are expected to yield accurate non-invasive blood flow quantification by NMR/MRI and to provide accurate simulation of signals/images under most schemes of MRI with and without flow of spins. We also show that in NMR/MRI under resonance conditions, magnetization and signals depends both on rf B1(x,t) and its spatial gradient for flowing spins, whereas for static tissue, they depend on rf B1(x,t) only, apart from T1, T2, proton density etc.. Using the master equations, new equations have been derived to describe accurately the final magnetization and signals resulting from static tissue under free induction decay, spin echo excitation and the methods can be extended to other schemes of excitations. We describe method for transformation of the equation to laboratory frame of axes.The new equations are expected to advance the MRI measurement techniques of parameters of clinical importance concerning tissues and blood flow. The paper presents challenges to Mathematcians on solutions of such equations, which will greatly advance the applications of MRI in medical science.

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4-HNE mediated TRPV1 dysfunction leads to loss of capsaicin-mediated eNOS activity and microvascular dysfunction

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Our lab has previously demonstrated enhanced 4-hydroxynonenal (4-HNE) post-translational modification (PTM) of TRPV1 decreases TRPV1 functional expression and contributes to microvascular dysfunction in diabetes. Furthermore, recent studies have shown TRPV1 activation elicits eNOS activation and NO production. Accordingly, we hypothesize that the enhanced 4-HNE-mediated PTM of TRPV1 contributes to diabetic microvascular dysfunction in db/db mice via decreased eNOS activation and resultant signaling. Contrast echocardiography demonstrated that 4-HNE decreased capsaicin mediated increases in myocardial blood flow and decreased capsaicin-mediated relaxation in isolated coronary microvessels. TRPV1 functional analysis using calcium imaging revealed blunted capsaicin-mediated calcium influx in the presence of 4-HNE in isolated mouse coronary endothelial cells (MCECs). Finally, 4-HNE treatment of MCECs decreased eNOS phosphorylation at Ser1197 leading to decreased measure in capsaicin-mediated nitrate production. Single point mutation, of a pore region cysteine residue 621, rescued channel activity and preserved capsaicin-mediated eNOS phosphorylation and NO production. 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 a signaling pattern aberrant in TRPV1 regulation of vascular control in diabetes.

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Centrosome amplification promotes cellular dedifferentiation to produce high-risk cancers

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The centrosome, consisting of a pair of orthogonal centrioles surrounded by a proteinaceous pericentriolar material (PCM), is the major microtubule organizing center in animal cells. Centrosome amplification (CA) occurs in diverse human cancers and can produce aneuploidy and chromosomal instability (CIN), common characteristics of cancer. We analyzed centrosome abnormalities in 362 breast cancer samples by probing a tissue microarray for pericentrin and polyglutamylated tubulin,

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representing PCM and centriole markers, respectively. A tumor was considered to have CA if the mean number of pericentrin foci per cell exceeded 2. CA is found in 35.1% of breast tumors and is associated with worse overall and recurrence-free survival. CA is found in 61.4% of triple negative breast cancers and less frequently in HER2-positive (41.2%) and hormone-sensitive/HER2-negative subgroups (29.2%). We hypothesized that CA could arise either de novo or by cell-doubling events (i.e. cytokinesis failure or cell-cell fusion). To evaluate the relative contributions of these two mechanisms, we correlated CA with chromosome content as determined by 6-chromosome centromeric FISH. There was a strong positive correlation between CA and ploidy, suggesting that CA most commonly arises from cell-doubling events. Additionally, we found a strong positive correlation between CA and CIN, suggesting that CA is a major cause of CIN in human cancer. CA was found in 54.8% of high-grade (grade 3) tumors, characterized by pleomorphic nuclei, mitotic figures, and lack of gland/tubule formation. To determine if CA induces high-grade (de-differentiated) phenotypes, we used an existing model of inducible CA in RPE1 and MCF10A cells. MCF10A cells with CA demonstrated decreased CD24 and increased CD44 expression, consistent with a more dedifferentiated phenotype. RPE cells with CA demonstrated increased expression of cytokeratins 7 and 19, also indicative of a more dedifferentiated state. Lastly, using a novel microfluidic device to simulate a mammary duct, MCF10A cells with CA were less likely to polarize and form ducts, also indicative of a dedifferentiated state. We conclude that CA is common in breast cancer, is commonly caused by genome doubling, and confers worse clinical outcome by inducing high-grade tumor phenotypes.

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A rat model for folate receptor antibody-mediated behavioral deficits: Implications of folate receptor autoantibodies in autism

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This study examines the role of antibodies against the folate receptor alpha (FRa) on brain development and function to determine if a similar mechanism could operate in autism spectrum disorders (ASD) and other neurodevelopmental disorders. A previous study in our laboratory found that >70% of children with ASD are positive for serum FR-autoantibodies. Consequently, we developed a rat model to study the behavioral and cognitive deficits induced by exposure to FR-antibody during gestation and weaning. We focused particularly on behavioral deficits that mirror those seen in ASD. We studied the transfer of FR-antibodies from mother to fetus and identified regions of the brain affected most by this antibody accumulation, which could potentially lead to the identification of regions involved in the functional and behavioral changes seen in ASD. Effective interventions to help in the treatment of those with ASD and FR-antibodies were considered, looking specifically at the efficacy of folinic acid and dexamethasone in preventing the behavioral deficits induced by FR antibodies in the rat model. Our results demonstrate that rats exposed to FR antibodies during gestation or weaning display core ASD symptoms such as deficits in communication, socialization, and set-shifting tasks. They also have learning and memory deficits. Treatment with folinic acid and dexamethasone results in significant improvement of the deficits. FR antibody exposure during gestation decreases folate transport from mother to fetus with antibody localizing to embryo, placenta, uterine wall, yolk sac, and amnion. Overall, these studies were aimed at understanding the effect of FR antibodies on fetal and infant brain development and function. The outcome of these studies could herald a paradigm shift in our understanding of ASD and other developmental disorders associated with FR autoimmune disorder.

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HNF1A-MODY diabetes in the human stem cell-derived hepatocyte-like cell

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HNF1A-MODY is the most prevalent form of monogenic diabetes and results from a haploinsufficiency of the transcription factor hepatocyte nuclear factor 1 alpha (HNF1A). The molecular mechanisms resulting in a diabetic phenotype remain unclear as haploinsufficient mouse models of HNF1A-MODY have failed to produce any phenotypes. In addition to its critical role in the pancreatic islet, HNF1A is known for its prominent role in regulating hepatocyte gene expression. We hypothesize that a subset of the pathophysiology of HNF1A-MODY results from gene expression changes in the hepatocyte. This original research relies on a model that allows for the directed differentiation of hepatocyte-like cells from human induced pluripotent stem cells (hiPSCs). A novel method for the selection of fully reprogrammed hiPSCs from the reprogramming milieu was established using a recombinant fusion E-cadherin and IgG cell culture matrix. Multiple hiPSC lines were generated from two novel HNF1A-MODY patients: one with a R272H mutation in the DNA-binding (a previously described mutation) and the other with a novel T564I mutation in the transactivation domain. These hiPSC clones express pluripotency factors by real time qRT-PCR, immunostaining, and flow cytometry to similar levels as control hPSCs. The HNF1A-MODY hiPSCs are capable of directed differentiation to the mature hepatocyte-like cell at similar efficiencies found for control hPSCs as measured by immunostaining and real time qRT-PCR for a variety of markers of the hepatic lineage. To validate consistency of samples from multiple sample lineages, a novel method was developed to screen for the selection of biological replicates of hepatocyte-like cells most similar in differentiation efficiency by real time qRT-PCR. Most experiments relied on 3 control genetically distinct hPSC lines and two separately maintained hiPSC lines per mutation genetic background for a total of 7 hPSC lines.

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Microarray analysis identified genes whose expression was affected in HNF1A-MODY hiPSC–derived hepatocyte-like cells at ≥2-fold difference in expression with p<0.001 using Partek Genomics Suite. Validation of all targets similarly affected in both genetic backgrounds for HNF1A-MODY with real time qRT-PCR identified three targets reliably and consistently differentially expressed between both HNF1A-MODY genetic backgrounds: AMDHD1, GSTT2, and IL1R2. Using HNF1A-MODY hiPSCs overexpressing wild-type HNF1A, IL1R2 expression could be induced specifically. Future work will confirm a functional decrease at the protein level of IL1R2, a decoy receptor for IL-1 signaling, and will functionally assess the potential contribution of a reduction in IL1R2 expression to a phenotype in HNF1A-MODY.

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Snapshot of MD.PhD Training in Canada: Structure, Finances, Mentorship and Outcomes

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Since the 1980s the Canadian Institute for Health Research (CIHR) has been funding MD/PhD programs, with most recent support at $2.4 million CAD per year. Until this year, CIHR has provided funding support for approximately 20 incoming students annually for 6 years. Unfortunately, the Canadian government has decided to discontinue funding as of 2016. Likely contributing to this decision is a lack of national outcome measures on the success of MD/PhD graduates. For this reason, CITAC (Clinician Investigator Trainee Association of Canada), a national student-led organization that represents the interests of over 500 trainees in Canada's Clinician Investigator training programs, including MD/MSc, MD/PhD, and Post-graduate clinician-investigator (CI) programs has begun to gather information on the demographics of Canadian CI trainees and alumni across all medical schools in Canada. Our preliminary analysis has shown that the structure and funding of MD/PhD programs varies greatly across Canadian schools with no data comparing the effectiveness the program structures. Moreover, we identified discordance between the reported mentorship needs of trainees and the perceived needs by program directors. Finally, data from the University of Toronto, University of British Columbia and McGill show than more than 65% of graduates continue research careers. Most notably, 90% of McGill's MD/PhD graduates (1989-2015) who responded to the survey (n=30 out of 41 contacted) reported holding Principal Investigator status on a grant, with 78% holding multiple grants and having an average h-index of 18 (well above the average in science of 10).

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MicroRNA-142 Regulates Eosinophilic Inflammation in Allergic Disease

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Rationale: MicroRNA (miRNA) are regulators of gene expression that have been shown to be important in hematopoiesis as well as allergic diseases, such as eosinophilic esophagitis (EoE), an allergic disease of the esophagus characterized by eosinophilic inflammation. We identified miR-142-3p and miR-142-5p, known hematopoietic miRNAs, among the top upregulated miRNAs in esophageal biopsies from patients with active EoE. Thus we hypothesized that miR-142-3p/5p were important for eosinophilic inflammation in allergic diseases. **Methods:** We quantified baseline eosinophil levels in the blood of wild type (WT) and miR-142-3p/5p-deficient (miR-142 KO) mice by flow cytometry, Discomb's fluid, and Diff-Quick staining. Next, using a modified Aspergillus fumigatus model of allergic lung disease, we characterized bronchoalveolar lavage fluid (BALF) and lung eosinophil levels in WT and miR-142 KO mice by flow cytometry and major basic protein (MBP) staining, respectively. Finally, we used ex vivo eosinophil bone marrow cultures and characterized eosinophil development in WT and miR-142 KO mice by cell counts and flow cytometry. **Results:** At baseline, miR-142 KO mice have decreased eosinophil levels in blood by flow cytometry (4.6-fold, $P < 0.001$), Discomb's fluid (12-fold, $P < 0.01$), and Diff-Quick staining (7.4-fold, $P < 0.05$) compared to WT mice. MiR-142 KO mice had decreased BALF cell counts (8.6-fold, $P < 0.001$), BALF eosinophil levels (37-fold, $P < 0.05$), and lung eosinophil levels (8.2-fold, $P < 0.0001$) compared to WT mice. MiR-142 KO ex vivo bone marrow cultures had increased total cells at day 4 (2.3-fold, $P < 0.001$), but decreased eosinophils at day 14 (1.7-fold, $P < 0.001$) compared to WT mice. **Conclusion:** Taken together, these data show that loss of miR-142-3p/5p resulted in decreased peripheral eosinophils at baseline and attenuated eosinophilic inflammation in a mouse model of allergic lung disease. Finally, these changes may be caused by impaired eosinophil development in miR-142 KO mice.

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Prevention of fibrosis delays healing in a mouse model of Systemic Lupus Erythematosus

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Objective: The purpose of this study is to examine the mechanism of delayed wound closure in a mouse model of Systemic Lupus Erythematosus (SLE). **Background:** SLE is a common and devastating autoimmune disease with symptoms including skin changes and poor wound healing. Specifically, wounds in SLE patients are often slow to heal and lead to fibrotic scars. Quercetin is an anti-fibrotic agent that also modulates immune cell activity. This study demonstrates that, in an SLE

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model, prevention of fibrosis delays wound healing and that this process may depend on lymphocytes at the wound site. **Hypothesis:** Anti-fibrotic treatment will unmask cell-mediated wound healing deficits in SLE. **Methods:** All animal experiments were approved by the Baylor Scott & White and Texas A&M Animal Care and Use Committees. Fas-deficient (LPR), and Fas-Ligand mutant (GLD) mice served as the SLE models. These mice were wounded with an 8 mm biopsy punch, treated daily with intraperitoneal injections of quercetin, and compared to wild-type quercetin-treated mice. Wounds were measured with calipers and sections of the wounds were stained with Masson's Trichrome and analyzed. Lymphocyte frequencies were measured by flow cytometry in the draining lymph nodes, skin, and wounds. Stains characterizing lymphocytes were CD3, the signaling complex associated with T-cell receptors and found on all T cells, CD4, the T-cell-associated receptor found on T-helper cells, and CD8, the T-cell-associated receptor found on cytotoxic T-lymphocytes. **Results:** Quercetin administration to LPR and GLD mice delayed wound healing compared with wild-type mice. On trichrome stain, LPR mice had decreased granulation tissue at the wound compared to WT and quercetin administration decreased fibrosis but did not increase granulation tissue accumulation. At day 10 post-wounding, LPR and GLD mice had decreased percentages of both T-helper (CD3+CD4+) and cytotoxic T (CD3+CD8+) lymphocytes in the draining lymph nodes of the wounds compared to wild-type animals. This decrease in T-cells may prevent adequate cytokine production at the wound site. Furthermore, CD4-CD8- lymphocytes expressing high levels of CD25, an Interleukin-2 receptor component, accumulate in LPR and GLD mice, and may modulate wound healing. **Conclusions:** Quercetin impairs wound healing in SLE-model mice, possibly by preventing fibrosis that normally compensates for immune-mediated wound healing deficits. The use of this anti-fibrotic agent unmasks a possible lack of cell-mediated healing in SLE, which is likely due to immune over-activation. This study therefore describes a novel model for understanding impaired wound healing in SLE and identifies lymphocytes as a potential treatment target for impaired wound healing in patients with SLE.

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A novel role for Plexin A in photoreceptor axon targeting in Drosophila

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Normal nervous system functioning is dependent upon very defined and precise network formation during development. Aberrant synaptic connectivity caused by mutations in axon guidance molecules and cell adhesion proteins has been associated with neurodevelopmental and psychiatric disorders such as intelligence disability, epilepsy, Autism Spectrum Disorders, and schizophrenia. The Drosophila visual system is an excellent model system for studying the basic mechanisms of axon pathfinding and neural circuit formation. The terminals of R7 and R8 photoreceptors, responsible for color vision, are segregated into distinct target layers of the medulla, a central region of visual processing in the brain. Knocking down a plexA in the eye and brain using RNAi resulted in R7 photoreceptors that prematurely terminate in the medulla and fail to expand their axon terminals. The plexA mutation, plexAMb0949, caused by a Minos element insertion in a coding exon causes a similar targeting phenotype in late pupae. PlexA protein is localized to processes just distal to the R7 termination layer in the medulla, suggesting that it might be an attractive signal for R7 axons, but it is also expressed in the photoreceptors themselves. We plan to perform a mosaic analysis of plexA in adults to determine which cells require PlexA. Overexpression of PlexA in photoreceptor axons results in hyperfasciculation of photoreceptor axons and premature termination. We will use this misexpression phenotype to characterize the mechanism by which PlexA mediates attraction or adhesion. One reported splice form of PlexA lacks the Semaphorin-1a-binding Sema domain. We are using overexpression and genome editing to test whether the Sema domain is necessary for PlexA function in the visual system. The same methods will be used to study the role of the cytoplasmic domain in order to determine whether PlexA acts as a receptor or a ligand in this context. Lastly, the uncharacterized gene CG6024 encodes a protein containing low-density lipoprotein (LDL) receptor class A repeat and CUB domains, which act as Plexin-binding domains in Neuropilins. Knocking down CG6024 in the eye caused a premature targeting phenotype in R7 cells similar to the plexA phenotype. Its cell-autonomous role in R7 axon guidance and possible relationship to plexA will be explored further, using CRISPR/Cas9 to generate a null mutant.

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Web Toolkit for the Integration of Molecular and Clinical Phenotype Data from a Panel of Human Glioblastoma Multiforme Xenolines

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Glioblastoma (GBM) has been intensely investigated for over 75 years and described in over 5,500 publications in the last 5 years representing 1% of all publications from the MeSH category of 'neoplasm'. Despite this the 5-year survival post-diagnosis remains at around 10%. It is theorized that the dismal clinical results are in part due to limitations in preclinical research models, namely immortalized cell lines that do not accurately represent clinical disease. To address these problems investigators have turned to patient-derived xenografts (xenolines) with primary tumor cells that have been solely passaged in immunocompromised mice that better reflect the molecular and phenotypic characteristics of human disease. Recently, a UAB cancer consortium molecularly characterized a panel of 27 human GBM xenolines at the level

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of the genome, transcriptome and kinome. Preliminary analysis of this data indicates that the xenoline tumors demonstrate molecular diversity across the samples and closely resemble the molecular phenotypes found in human tumors. Moreover, a well-characterized GBM xenoline system is a testable and clinically translatable model system. However, the large amounts of molecular and phenotype data remain difficult to analyze without significant and transparent knowledge of the research methods, and data analysis techniques utilized across the numerous labs that generate the data. To correct this and eventually allow community access to these datasets, we have begun creating an annotated, queryable database that allows access to the three major clinical, molecular and phenotypic data associated with these GBM samples. This web-based interface for interacting with the data allows for simple queries to be run without significant knowledge of the database structure. Along with this we have created tools for basic visualization and downloading of the data retrieved from the query itself. Additionally the modular design allows the system to accommodate any number of analytical methods as analyses become more complex. While the development of this platform has just begun, our preliminary data indicate these models represent human tumors with a much higher fidelity than cell lines. We believe that the creation of this database and its future integration with GBM data from The Cancer Genome Atlas (TCGA) will allow for in silico predictions of drug response that can then be directly tested in xenoline tumor model and furthermore can be translated to better patient care.

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Role of scaffolding protein IQGAP[1] in fat metabolism

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IQGAP1 (IQ motif-containing GTPase Activating Protein 1) is a ubiquitously expressed protein that is known to integrate signaling from various cellular processes including cellular motility, adhesion, and proliferation. Furthermore, IQGAP1 has been shown to modulate MTORC1 signaling, indicating an important role for this protein in metabolism. These findings reveal that IQGAP1 may integrate proliferation and metabolism. Since its role in proliferation is characterized, we examined the metabolic impact of IQGAP1. We found that fasting induces IQGAP1 levels in the liver; therefore, we analyzed the fed and fasted response of wild-type and Iqgap1-/- mice. During the fed state, excess energy is converted to free fatty acids in the liver and is typically stored in adipose tissue. Compared to wild-type, Iqgap1-/- mice exhibit decreased expression of fatty acid synthase. Consequently, the fed Iqgap1-/- mice displayed reduced serum triglyceride levels and white adipose tissue compared to wild-type mice. These findings clearly indicate that loss of IQGAP1 results in dysregulation of lipid metabolism. To further characterize the role for IQGAP1 in regulating lipid metabolism, we fasted wild-type and Iqgap1-/- mice and examined their response. As expected, fasting resulted in a decrease in the liver to body weight ratio and serum triglycerides in both sets of mice. But surprisingly, Iqgap1-/- mice had reduced white adipose tissue and displayed a blunted induction of the beta-oxidation genes. This suggests that Iqgap1-/- mice have a putative decrease in fatty acid-derived acetyl-CoA flux into the ketogenic pathway. It is known that in ketogenic conditions, such as fasting, increased expression of beta-oxidation genes is dependent on liver-derived factor FGF21, which is secreted into the serum. Therefore, we analyzed Fgf21 expression and found that its induction upon fasting in Iqgap1-/- mice is significantly blunted compared to wild-type. This was also consistent with the circulating serum FGF21 levels. Next, we examined the response of Iqgap1-/- mice to ketogenic diet. After 4 weeks on ketogenic diet, Iqgap1-/- mice had a higher liver to body weight ratio but a dramatically lower white adipose tissue to body weight ratio, suggesting an altered distribution in energy and metabolism occurs in the absence of IQGAP1. Moreover, these mice had reduced expression of Fgf21, Acadm, Ehhadh, and the rate-limiting enzyme for ketone body synthesis, Hmgcs2. Excitingly, the in increase in serum ketone bodies found in wild-type mice on ketogenic diet was completely absent in Iqgap1-/- mice. Overall, our findings reveal that IQGAP1 is crucial to regulate lipid metabolism as well as mediating the ketogenic response.

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The Dynamic Role of MARCKS in the Progression and Resistance of Glioblastoma

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Background: Glioblastoma multiforme (GBM) is the most common and deadly form of Glioma, with a median survival of 14 months. 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; where-as Myristoylated Alanine Rich C-Kinase Substrate (MARCKS) electrostatically sequesters PIP2. It has been shown that activating mutations of PI3K[3], deactivating mutations of PTEN [4] and reduced levels MARCKS all correlate with a worsened GBM patient survival.GBM's frequent deregulation of the RTK/RAS/PI3K pathway, MARCKS ability to starve this pathway of the substrate needed to drive cell growth, survival and migration, as well as MARCKS beneficial presence in the proneural subtype with unmethylated MGMT promoter, demonstrate the necessity of understanding the mechanism by which MARCKS impacts GBM progression and treatment sensitivity. **HYPOTHESIS:** MARCKS is a key regulator

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of GBM growth and sensitivity, therefore, increasing levels of unphosphorylated MARCKS in combination with DNA damaging Temozolomide (TMZ) therapy 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 a cell line that expresses 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). MARCKS effect on cell growth and signaling, clonogenic potential, DNA damage repair, and radiation and chemotherapy sensitivity were analyzed. **Results:** Increasing WT and NP MARCKS levels resulted in significant tumor growth suppression.

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Spns2 is required for effector lymphocyte migration during an immune response

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Sphingosine 1-phosphate (S1P) is a signaling lipid that plays critical roles in a wide range of diseases including multiple sclerosis, asthma, and colorectal carcinoma. FTY720, which targets four of the five S1P receptors, has been FDA-approved for treatment of multiple sclerosis. By targeting S1P receptor 1 (S1PR1) on T cells, FTY720 blocks T cell exit from lymph nodes, where they are initially activated, and prevents them from travelling to sites of inflammation. FTY720 and similar next-generation drugs have also been extremely promising in the treatment of an array of inflammatory diseases and cancers. Unfortunately, this class of drugs also targets S1P receptors throughout the cardiovascular system, where S1P signaling regulates endothelial and cardiac function via S1PR1. For example, FTY720 has been shown to modulate S1P signaling on vascular endothelial cells and cardiomyocytes, causing macular edema and dangerous bradycardia in patients. It has been a long-standing goal to develop strategies to manipulate lymphocyte trafficking while sparing the vasculature and the heart. We recently found that the transporter SPNS2 is required to supply lymph S1P, which guides T cell exit from lymph nodes, but does not play a major role in maintaining the levels of blood S1P required for normal vascular and cardiac function. As the first known protein that differentially regulates lymph versus blood S1P, SPNS2 may be an attractive target for immune suppressive drugs that block T cell trafficking while limiting cardiovascular side effects. It is unknown, however, whether SPNS2 continues to be essential for lymphocyte egress from lymph nodes during inflammation, or whether other S1P transporters are up-regulated and compensate for SPNS2's loss. Here we found that effector T cells in SPNS2 deficient animals do not accumulate in the skin in a mouse model of dermal inflammation, despite T cell activation and proliferation within the draining lymph node, suggesting an exit block. Moreover, in a mouse model of multiple sclerosis (experimental autoimmune encephalomyelitis, EAE), inducibly deleting Spns2 in adult animals using Ubc-CreERT2 protects animals against disease, providing further evidence that Spns2 may be a promising drug target for autoimmune diseases.

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Implications of endocytic vesicle rupture for cell-to-cell transfer of alpha-synuclein aggregates in Parkinson's disease

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Numerous recent studies have established the ability of aggregated alpha-synuclein (a-syn) to spread from cell-to-cell in a prion-like fashion, whereby the propagation of both its misfolded conformation and higher-order aggregation state from affected neurons to neighboring cells constitutes a central mechanism of pathological progression in Parkinson's disease. Understanding the cellular and molecular forces responsible for this transfer between cells is critical to developing treatment approaches designed to arrest or prevent disease progression. In the circular pathway of a-syn cell-to-cell transmission, our lab has addressed the previously unexplained phenomenon of endocytic vesicle exit by demonstrating that a-syn aggregates are able to induce lysosomal rupture, a type of cellular damage that causes cathepsin-mediated oxidative stress and inflammasome activation. Further characterization of this disruptive cellular entry mechanism has resulted in our subsequent demonstration that aggregates of both WT and familial mutant a-syn are capable of significantly inducing vesicle rupture as measured by mCherry-galectin-3 (chGal3) relocalization in a human neuroblastoma cell line. Moreover, we show that differences in a-syn aggregate conformation as present in structurally well-defined a-syn strain assemblies result in different levels of vesicle rupture potency, implicating vesicle rupture as a mechanism for strain-specific differences in target cell pathology. Importantly, we have also validated a-syn-induced vesicle rupture with our chGal3 model using mature dopaminergic neurons derived from induced pluripotent stem cells. Because consequences of vesicle rupture like lysosomal/autophagic dysfunction and oxidative stress are known driving forces behind release of a-syn aggregates from an affected cell, we additionally investigated cell-to-cell transfer of a-syn in the context of aggregate-induced vesicle rupture by utilizing live cell fluorescent imaging. Ruptured vesicles containing a-syn can be observed in the extracellular environment, and can be seen trafficking from an affected cell to a neighboring healthy cell. These results implicate a-syn induced vesicle rupture as an important consequence of endocytic entry, and suggest that this form of cellular damage can serve as both a driving force and a vector for a-syn release and subsequent transmission to neighboring neurons.

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A novel mechanism of neuro-endothelial crosstalk in the pressor response to acute behavioral stress

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The normal physiological reaction to acute behavioral stress is well established to include a rapid increase in sympathetic output that mediates an acute pressor response. More recent studies have demonstrated that acute behavioral stress induces an increase in plasma endothelin-1 (ET-1) in both humans and animal models. However, both the cellular source of this ET-1 release and its downstream functional consequences have yet to be determined. We hypothesized that acute behavioral stress mediates endothelial release of ET-1, which contributes, in part, to the subsequent acute pressor response. Adult male vascular endothelial-specific ET-1 knockout (VEETKO) and flox control mice were utilized for all experiments. Aortic and renal vascular preproET-1 mRNA expression was significantly reduced in VEETKO compared with flox mice (1.0 ± 0.2 vs. 0.03 ± 0.01 A.U. and 1.0 ± 0.2 vs. 0.3 ± 0.1 A.U. respectively, $p<0.05$). Acute behavioral stress was experimentally induced by utilizing the established cage switch stress (CSS) model. In control mice, plasma ET-1 was significantly elevated in response to CSS (0.92 ± 0.04 vs. 1.2 ± 0.08 pg/ml, $p<0.05$), whereas in VEETKO mice, CSS did not elicit an increase in plasma ET-1 (0.52 ± 0.09 vs. 0.63 ± 0.08 pg/ml, $p>0.05$). Blood pressure, heart rate, and activity were measured in conscious freely moving mice by radiotelemetry. In both VEETKO and control mice, CSS induced an acute pressor response. However, the pressor response in the 10 minutes following CSS was significantly blunted in VEETKO compared to control mice, as indicated by mean arterial pressure (Interaction: $p<0.001$, Genotype: $p<0.05$), systolic pressure (Interaction: $p<0.001$, Genotype: $p<0.05$), and diastolic pressure (Interaction: $p<0.001$, Genotype: $p<0.05$). The CSS induced response was similar between genotypes for pulse pressure, heart rate, and activity. VEETKO and flox mice demonstrated a similar reduction in adrenal norepinephrine (NE) content in response to CSS, indicating similar CSS mediated sympathetic activation between genotypes. Importantly, pulmonary ET receptor expression was similar in both genotypes, indicating no difference in ET-1 clearance capacity between groups. These results indicate that endothelial ET-1 release contributes the pressor response to acute behavioral stress, thus identifying a novel mechanism of neuro-endothelial crosstalk that contributes to this important physiological reaction. Additionally, these findings may have implications to the mechanistic connection between chronic exposure to stressful events and the development of cardiovascular disease. This work was supported by funding from the NIH - P01 HL095499 and P01 HL069999 to Dr. Jennifer Pollock and Dr. David Pollock, F30 DK107194 to Brandon Fox, and MSTP T32 GM008361.

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The anti-cancer effects of targeting Mer and Axl in preclinical models of colorectal cancer

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Background: The TAM (Tyro3, Axl, Mer) family of receptor tyrosine kinases (RTKs) have emerged as oncogenic drivers in many malignancies when aberrantly expressed. Mer and Axl promote cancer cell survival, metastasis and chemoresistance by activating well-characterized oncogenic pathways and attenuating anti-tumor immune responses. Recent data from our lab suggest that a subset of colorectal cancer (CRC) cell lines are sensitive to the experimental Mer-selective tyrosine kinase inhibitor, UNC2025, at nanomolar doses. This indicates potent activity and the ability to optimize outcomes for a range of patients. Thus, our Specific Aims were: 1. Test the efficacy of UNC2025 in combination with approved therapies in CRC cell lines.. 2. Develop novel cell lines with genetic knockdown or overexpression of Mer and Axl to validate UNC2025 specificity. 3. Assess potential crosstalk between Mer and Axl. **Methods and Results:** The combination of UNC2025+Trametinib in K-ras mutant cell line GP2D and UNC2025+Erlotinib in K-ras wildtype cell line SW48 resulted in synergistic inhibition of cell proliferation. Immunoblots demonstrated a treatment-induced decrease in downstream oncogenic signaling. This corresponded with a significant increase in apoptosis as assessed by annexin staining and flow cytometry. CRC cell lines HCT116 and GP2D were transduced to create a Mer ORF (open-reading frame; overexpressor) and an Axl ORF cell line, respectively. A CRISPR/Cas9 construct was used to create a GP2D Mer knockdown cell line. Post-treatment, the GP2D Axl ORF cell line was more chemoresistant than the wildtype cell line, while Mer CRISPR was less resistant than wildtype, as assessed by Cell TiterGlo. Colony formation was assessed post-drug treatment using clonogenic assays; in these experiments, overexpressing Mer conferred chemoresistance. **Conclusions:** Mer and Axl may be effective anti-cancer targets in CRC. The synergy observed with the two pathway-targeting agents suggests a promising therapeutic paradigm. These results validate the continued development of drugs that target TAM family RTKs.

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Interferon gamma inhibits cognitive recovery from West Nile Virus

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West Nile Virus is the leading cause of arboviral encephalitis in the United States. Despite high survival rates, many patients experience persistent neurocognitive deficits such as memory

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loss, depression or fatigue, for which there is no treatment or specific therapy. Little is known about how the neuroimmune interactions that help to clear viral infection from the CNS may contribute to long-term cognitive recovery. We hypothesize that interferon gamma, a key inflammatory cytokine important in viral clearance that is also known to modulate neurogenesis, may be contributing to delayed cognitive recovery. Using a novel murine recovery model of WNV encephalitis developed in our lab, we performed cognitive testing on the Barnes Maze, 45 days following infection with WNV-E218A. We found that mice lacking interferon gamma signaling (IFNgR-/-), were protected from visual-spatial learning deficits compared to wild-type mice (C57BL/6J), despite having similar numbers of immune infiltrates, delayed viral clearance, and a more severe clinical course. These studies suggest that interferon gamma inhibits cognitive recovery and help to validate the translational possibility of interferon gamma as a therapeutic target for the many patients experiencing delayed cognitive recovery from WNV.

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Effector CD4+ T cell subsets employ distinct programs for motility in the inflamed dermis

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The effector function of CD4 T cells is critically dependent on the ability of T cells to rapidly enter and traverse a wide variety of peripheral tissues to survey for damage, locate foci of infection, or cause pathology from chronic infection or autoimmunity. Although the processes of homing to and entry into inflamed tissues have been characterized, the factors that drive and regulate interstitial motility remain undefined. It has been widely assumed that T cells migrate independently of underlying adhesions to the extracellular matrix and guided by cues from extracellular chemokine gradients. However, using intravital multiphoton microscopy, we have shown that both Th1 and Th2 cells are dependent on aVb1/b3 (alphaV beta1/beta3) integrins for motility along ECM fibers in a model of microbial inflammation. While both cell types require integrins for motility, a fine analysis of Th1 and Th2 dynamics in the inflamed dermis showed that Th1 cells were more frequently arrested and exhibited a decreased area of surveillance in comparison to Th2 cells, suggesting other differences in the regulation of motility. An analysis of motility-associated genes revealed several genes that were regulated distinctly in Th1 and Th2 cells, including b3 (beta3) integrin. While b3 integrins are essential for the interstitial motility of both Th1 and Th2 cells, flow cytometric analysis confirmed that Th2 cells express higher levels of b3 (beta3) integrin in both the draining lymph node and inflamed dermis. As integrins can be activated directly via ligand binding, or through inside-out signals downstream of G protein-linked chemokine receptors, we hypothesized that increased expression of integrins on Th2 cells may bypass a need for chemokine signals. Indeed, the motility of Th1, but not Th2 cells, was strikingly sensitive to inhibition of Gai (G-alpha i) or Gbg (G beta-gamma) signaling. We further demonstrated that Th1 motility was particularly sensitive to the blockade of CXCL9 and CXCL10, suggesting a critical role for CXCR3 in the interstitial motility of Th1 cells. Additionally, in the absence of chemokine signals, the continued motility of Th2 cells remains integrin-dependent, suggesting that the integrin-mediated motility of Th2 cells occurs in the absence of inside-out signaling. We hypothesize that a requirement for chemokine signals to induce motility in Th1 cells provides an additional level of spatial regulation in the inflamed skin. Ultimately, exploiting differences in migration could provide for more specific therapy to inhibit inappropriate immune responses without disturbing effective protection against pathogens.

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Investigation of Rad^5 function in error-free PRR

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Mistakes occurring in DNA replication can result in mutations leading to cellular dysfunction, manifesting as cancer and various diseases. While the cell is capable of excising and repairing damaged DNA, these mechanisms are sometimes bypassed, allowing regions of damaged DNA to persist in the genome. These lesions pose a major problem for replication machinery. The replication fork will stall at sites of DNA damage, putting the cell at risk for chromosomal rearrangements and double stranded breaks that often result in cell death. To prevent such catastrophic outcomes, the cell utilizes damage-tolerance pathways that allow for continued DNA replication through regions of damage. One such pathway is known as error-free post-replication repair (PRR). Error-free PRR relies upon the activity of the Rad5 protein. Unlike other damage-tolerance pathways, Rad5 rearranges DNA in such a way that regions of damaged DNA are not used as a template during the replication process. As a result, while other damage-tolerance pathways inaccurately add nucleotides across from sites of DNA damage (thus introducing disease-causing mutations), error-free PRR always leads to the incorporation of the intended nucleotide, thereby preventing the introduction of mutations. The goal of this research is to clarify the mechanism of Rad5 mediated DNA rearrangement in error-free PRR.To do so, the function of the Rad5 protein will be investigated and compared to that of Rad5 mutant proteins that have shown to be defective in ATPase activity. The DNA binding abilities of the Rad5 protein will be tested. In addition, ensemble experiments will be used to study the ATPase and helicase activity of the Rad5 helicase domain. Ultimately we will examine the ability of Rad5 to form Holliday junction intermediates, thus elucidating the mechanism by which Rad5 rearranged DNA contacts to carry out fork reversal. All together, we hope to clarify Rad5's role in error-free PRR. By better understanding the role of Rad5 in DNA damage tolerance, we aim to open the door for future manipulation of error-free PRR pathways in order to prevent potentially cancerous or otherwise harmful mutations.

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Automated fiber quantification of the fornix predicts outcome after surgery for intractable temporal lobe epilepsy

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Background and Purpose: The reasons for persistent postoperative seizures in patients with refractory temporal lobe epilepsy (TLE) due to hippocampal sclerosis (HS) remain unknown. In the present study, we reconstructed the fornix from DTI data and quantified regional tissue properties along the fornical pathway to investigate whether preoperative fornical tract profiles were related to postoperative seizure outcome in patients with refractory TLE. Non-invasive neuroimaging prognostic markers that stratify patients according to likely postoperative outcome would be extremely helpful for clinical decision making and preoperative counseling. **Methods:** We recruited 42 patients with TLE due to HS and 44 healthy age- and sex-matched controls into this study. All patients underwent comprehensive preoperative evaluation, preoperative MRI including 60-direction DTI, amygdalaohippocampectomy, and postoperative seizure outcome assessment. Controls received the same imaging protocol. We reconstructed the left and right fornix and generated fornical tract profiles from the DTI data for each participant using an extension of automated fiber quantification (AFQ) methods. Group-wise comparisons for mean diffusivity (MD) and fractional anisotropy (FA) were made between controls and patients with an excellent (ILAE 1) or suboptimal (ILAE 2+) postoperative seizure outcome using the standardized International League Against Epilepsy (ILAE) outcome classification. Statistical tests were performed using a two-sample t-test at a significance level of $\alpha = 0.05$. Multiple comparisons were corrected for using false discovery rate (FDR) thresholding with n = 30 total comparisons. **Results:** 22 (52%) patients were found to have an excellent postoperative seizure outcome and 20 (48%) had a suboptimal outcome. There were no significant differences in the FA tract profiles, but significant differences were detected in the MD tract profiles for both patient groups in the ipsilateral temporal lobe. Significant differences were also detected in patients with suboptimal surgical outcomes outside of the ipsilateral temporal lobe. **Discussion and Conclusion:** We observed that patients with a suboptimal postoperative outcome deviated from healthy controls and patients with an excellent outcome in tissue properties of the ipsilateral fornix extending outside of the temporal lobe. This abnormal tissue is located outside the margins of resection of conventional temporal lobe surgery, and may contribute to support a postoperative epileptogenic network. This extends recent findings indicating that thalamohippocampal pathways – that are largely mediated by the fornix – are preferentially abnormal in patients with persistent postoperative seizures. Abnormal tissue characteristics of the ipsilateral fornix outside the margins of resection may be a candidate imaging prognostic marker of continuing postoperative seizures in refractory TLE.

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Optimization of single-chain insulin stability for therapeutic use in the developing world

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Single-chain insulin analogs (SCIs) provide a platform technology for the development of degradation-resistant insulin therapeutics that can withstand harsh storage conditions without loss of biological activity and show promise to enhance access to Insulin Replacement Therapy for diabetes patients in technology-limited regions where refrigeration is scarce. This study utilizes structure-based design principles to identify and probe key determinants of thermodynamic and physical stability in an SCI template, designated SCI-biphasic, to guide the future creation of optimized SCI analogs. Several residues hypothesized to contribute to the thermodynamic and physical stability of SCI-biphasic were identified, mutated using site-directed mutagenesis, and expressed and purified from Pichia pastoris. Replacement of GluA14 with TyrA14 resulted in a loss of folded stability of ~1 kcal/mol whereas replacement of HisA8 with ThrA8 decreased thermodynamic stability by ~0.5 kcal/mol. These findings demonstrate that GluA14 avoids the entropic penalty incurred by solvent exposure of non-polar Tyr in WT insulin and that HisA8 enhances stability through improvement of the A1-A8 helical C-cap. Variant linker sequences of AEGPRR, EAGPRR, and EEGARR had negligible differences in stability relative to the parent EEGPRR linker in SCI-biphasic. The EEGARR linker variant showed similar activity and pharmacodynamics in diabetic rats as parent SCI-biphasic. These new insights will help facilitate the tuning of SCI stability via selective mutations at key residues.

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Development of an induced pluripotent stem cell model to study mechanisms of UBE^{3}A imprinting in Angelman syndrome

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Genomic imprinting is an epigenetic phenomenon in which genes are expressed in a parent-of-origin specific manner. The 15q11.2-13.1 locus is one such region in the human genome in which a number of imprinted genes are functionally haploid. UBE3A is a gene that codes for an E3 ubiquitin ligase, and it is solely expressed from the maternal allele of 15q11.2-13.1 in neurons. The paternal copy of this gene is intact, but remains silenced due to transcription of a nuclear-localized long non-coding RNA, UBE3A antisense transcript (UBE3A-ATS) in the direction opposite that of the paternal UBE3A. Maternal

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deficiency of UBE3A results in the neurodevelopmental disorder known as Angelman syndrome (AS). Children with AS present with severe cognitive dysfunction, absent speech, ataxic gait, and a characteristic happy affect. As all patients with AS carry at least one copy of UBE3A on the paternal allele of the 15q11.2-13.1 locus, reactivation of the silent paternal allele of UBE3A may ameliorate the severity of symptoms in patients with this disorder. Recent efforts focused on restoring the function of paternal UBE3A using mouse models of Angelman syndrome have identified topoisomerase inhibitors and antisense oligonucleotides (ASOs) directed against UBE3A-ATS as potential therapeutics. Both candidate therapies work by reducing transcription of UBE3A-ATS, indicating an important mechanistic approach for treating AS. Little is known about how UBE3A-ATS functions to silence paternal UBE3A, thus limiting attempts to refine the potential therapeutic approaches. Furthermore, like most lncRNAs, UBE3A-ATS sequence and structure is not conserved between mouse and human. Thus, the study of UBE3A-ATS function in relevant human tissues is necessary for the development of AS therapeutics. Induced pluripotent stem cell (iPSC)-derived neurons generated from AS patient fibroblasts provide a unique opportunity to further elucidate the interaction between paternal UBE3A and UBE3A-ATS in human neurons. We have previously shown that UBE3A-ATS is produced and paternal UBE3A is silenced after 10 weeks of neuronal differentiation in AS iPSCs. In order to study mechanisms of UBE3A-ATS function, we are generating a targeted insertion of an ectopic EF1-alpha promoter upstream of UBE3A-ATS on the paternal allele in AS iPSCs. We predict that this iPSC line will express UBE3A-ATS and silence UBE3A without 10 weeks of neuronal differentiation, thus facilitating the study of UBE3A-ATS function in stem cells. Future studies will focus on using enzymatically-inactive Cas9 (dCas9) coupled with transcriptional repressors to determine the extent to which UBE3A-ATS has to be transcribed in order to repress paternal UBE3A.

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Binding Extracellular Matrices in Aqueous Environments using Nanoparticle Solutions for Vascular Tissue Engineering

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Introduction: Robust binding of soft tissues in aqueous environments remains a challenge in both the clinical setting, for improving surgical outcomes, and for scaffold fabrication in tissue engineering applications. One approach to engineer tissues involves the development of a 3D structure, or scaffold, to support cellular infiltration and tissue regeneration. Our group is exploring the use of a planar extracellular matrix (ECM), derived from the amniotic membrane, to create multi-laminate scaffolds for a variety of tissue engineering applications. As a proof of principle, we are focused on investigating binding conditions of rolled planar amnion for blood vessel tissue engineering. A specific challenge in this application is the aggressive hemodynamic environment that can result in the graft layers delaminating. As such, an approach is needed to stabilize the ECM lamination without the use of toxic treatments. Silica nanoparticles (SNP) adsorb onto tissue at the macromolecular level; here we explore this characteristic of SNP to enhance the binding of a multi-layered, tubular ECM scaffolds. **Materials and Methods:** Monodisperse colloidal SiO2 nanoparticles 25 nm in diameter were purchased from Sigma-Aldrich and diluted with DI H2O to reach the desired concentration. Human amniotic membrane was first decellularized using 0.1% w/v SDS. Rectangular ECM sheets were treated with SNP at incremental surface densities of 8.75, 17.5, 35 or 70 µg/mm2, rolled and subsequently lyophilized to generate laminated tubular geometries. The mechanical properties of the scaffolds were assessed by tensile testing of ringlets for binding strength and mechanism of failure. Scaffolds were also tested for suture retention strength. Furthermore, myofibroblast cell cultures were incubated with a range of SNP concentrations for 12 hours to evaluate cytotoxicity of the nanoparticles. **Results and Discussion:** Results show that SNP-treated tubular ECM grafts maintain their shape upon rehydration, have improved tensile strength, and change the mechanism of failure from interfacial slipping to single-point fracture. This change in the mechanism of failure is a significant improvement for the use of this scaffold in vascular tissue engineering applications as the walls of the graft would experience pulsatile radial stress and thus the layers that comprise the wall must be able to withstand interfacial shearing forces. Preliminary investigation also shows cytocompatibility in 2D culture, even with direct exposure to nanoparticles at high concentrations. The ability to adhere hydrated soft tissue without compromise to biocompatibility would provide a means to layer ECM in order to develop scaffolds of more complicated and tunable geometries unlike traditional ex-vivo derived tissue scaffolds such as allografts or xenografts.

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MMP-13 is a critical driver of lu[illegible]uction in a viral exacerbati[illegible]

WITHDRAWN

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Introduction: Cigarette smoke directly induces the expression of matrix metalloproteinases (MMPs), which play an important role in lung destruction in emphysema. Viral infections have significant consequences in smokers, particularly in patients with COPD. Little is known about the regulation of the collagenase MMP-13 in the lungs under smoke-exposure and viral infection conditions. Here we explore the role for MMP-13 in emphysema development and COPD exacerbations. **Methods:** Bronchoalveolar lavage (BAL) fluid samples from FORTE trial participants with COPD, smokers without disease, and control

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patients were examined for MMP-13 levels by ELISA. MMP-13 serum levels from LEUKO trial participants were examined at the time of COPD exacerbation and after 30 days. Using an acute COPD exacerbation model, mice underwent two weeks of smoke-exposure followed by influenza virus infection (PR8 influenza virus) and were sacrificed 14 days later. A second, long-term smoke-exposure (12 months) was also performed. Lungs were pressure perfused with 10% formalin to 25 cm H2O. Tissue samples were paraffin embedded and step sectioned (4 μm). Slides were H&E stained and 40 images were obtained at random from at least 4 sections at 10x magnification. Mean linear intercept was calculated to quantify the amount of tissue destruction. Tissue was analyzed for MMP-13 levels by ELISA analysis. **Results:** In BAL fluid, MMP-13 protein concentration is elevated in patients with COPD as compared to non-smokers as well as smokers without disease. Serum samples from patients with COPD exacerbations demonstrate that as opposed to non-smokers, current smokers with COPD do not decrease serum MMP-13 levels after recovery from COPD exacerbation. In the murine model of COPD exacerbation, MMP-13 mRNA expression is upregulated in whole lung homogenates, and MMP-13 protein levels are increased in the lung and BAL fluid. While WT mice develop airspace enlargement in the COPD exacerbation model, MMP-13 KO mice develop attenuated emphysema. This is in contrast to mice exposed only to smoke for 12 months, where there is no difference in airway enlargement in the MMP-13 KO as compared to WT smoke-exposed animals. **Conclusions:** The present study demonstrates the importance of MMP-13 in COPD exacerbations and identifies this protease as a possible mediator of lung destruction following viral disease exacerbations. While loss of MMP-13 does not prevent lung destruction in chronic smoke-exposure conditions, it impacts airspace enlargement in the setting of smoke-exposure and viral infections. This suggests that targeting MMP-13 in the setting of virally induced COPD exacerbations may be a viable treatment option to prevent lung function decline.

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Tissue resident alveolar macrophages are lost during influenza a infection in mice

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Rationale: Influenza A pneumonia is the most common cause of death from an infectious agent and can become an important contributor to overall mortality during pandemics. Severe influenza A pneumonia results in the development of the acute respiratory distress syndrome, which is the proximal cause of death in most of these patients. It is now recognized that alveolar macrophages represent a heterogeneous population of cells. Tissue resident alveolar macrophages are long lived, self-renewing cells with developmental origins outside of the bone marrow. During infection, monocytes are recruited to the lung where they differentiate into alveolar macrophages. The role and fate of these different macrophage populations during the course of influenza A pneumonia is not known. Hypothesis: Influenza A infection results in the replacement of tissue resident alveolar macrophages with monocyte derived cells that persist after injury. **Methods:** We infected ten to twelve week old C57Bl/6 mice intratracheally with lethal and sublethal doses of influenza A/WSN/33. We analyzed inflammatory cell populations in lung digests at different time points after the influenza A infection using multi-color flow cytometry. We developed a shielded bone marrow chimera system to unambiguously identify tissue resident and alveolar macrophage populations during the development and resolution of influenza A infection. **Results:** In the early stages of influenza A infection, tissue-resident alveolar macrophages could be readily distinguished from monocyte-derived cells using flow cytometry. Tissue-resident alveolar macrophages were rapidly depleted during influenza A infection accompanied by an influx of monocyte derived cells into the lung. Using bone marrow chimeric mice generated with lung protection followed by myeloablation, we observed that monocyte derived cells persisted in the lung after the resolution of infection for as long as 10 months but could no longer be distinguished from tissue resident cells on the basis of flow cytometry. **Conclusions:** Influenza A infection results in the dose-dependent depletion of tissue resident alveolar macrophages in the lung. Whether this results from direct infection by the influenza A virus or secondary effects related to lung tissue destruction and inflammation is not clear. Monocytes recruited to the lung during influenza A infection differentiate into alveolar macrophages in the local microenvironment of the lung and persist long after the resolution of infection. Our results suggest that severe influenza infection results in permanent changes in tissue resident alveolar macrophage ontogeny.

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Interleukin-22Ra1 signaling attenuates intestinal inflammation and promotes intestinal stem cell differentiation

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Introduction: Necrotizing enterocolitis (NEC), a leading cause of death in premature infants, is triggered by an exaggerated inflammatory response resulting in intestinal necrosis. Previously we have shown that NEC-induced inflammation leads to gut barrier dysfunction, intestinal stem cell loss, and impaired mucosal healing. Recently, interleukin (IL)-22 signaling has proven to play a critical role in attenuating intestinal inflammation, maintaining the gut barrier and promoting intestinal wound healing. We sought to test whether IL-22 signaling protects against intestinal inflammation and determine the mechanisms involved. **Methods:** *In vitro* studies were performed on intestinal epithelial cell line (IEC-6), pre-treated with recombinant IL-22 (rIL-22) and lipopolysaccharide (LPS) stimulation. qRT-PCR for the pro-inflammatory cytokine IL-6

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and immunocytochemistry for nuclear NFkB translocation was performed with confocal microscopy. RNA sequencing was performed on the intestine of wild-type and mice deficient in intestinal IL-22 signaling (IL-22Ra1ΔIEC). Their paneth and goblet cells were evaluated via immunohistochemistry. Mice were treated with IgG or rIL-22 2x/wk from postnatal day 10-28. As a model of endotoxemia, neonatal mice were given LPS for 6 hr with IgG or rIL-22. **Results:** In IEC-6 cells, rIL-22 pretreatment reduced pro-inflammatory NFkB nuclear translocation and IL6 expression. RNA sequencing revealed mice deficient in intestinal IL-22Ra1 signaling have significant decreases in genes critical for intestinal stem cell development and a decreased number of goblet and paneth cells. Moreover, mouse pups treated with rIL-22 had decreased toll-like receptor 4 (TLR4) mRNA expression and increased paneth and goblet cells. Strikingly, mice that received rIL-22 and LPS had decreased IL-6 mRNA levels in their small intestine compared to pups that received LPS alone. **Conclusion:** IL-22 protects against inflammation in intestinal epithelial cells *in vitro* and *in vivo*. IL-22Ra1 signaling promotes intestinal stem cell development and regulates the number of goblet and paneth cells, an important aspect of gut barrier maintenance. Strikingly, mouse pups treated with rIL-22 demonstrated decreased TLR4 expression, suggesting that IL-22 may protect against TLR4-mediated diseases including sepsis and NEC.

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Endocytic Trafficking of Alpha Synuclein in Models of Parkinson's Disease

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The amyloid protein alpha synuclein is the main component of Lewy body structures, the histopathological hallmark of Parkinson's disease (PD). Compelling evidence suggests that toxic, misfolded aggregates of alpha synuclein are capable of spreading from affected cells to unaffected cells in a prion-like manner, explaining the progressive loss of dopaminergic neurons within the substantia nigra of PD patients. When exogenous aggregates of alpha synuclein bind the external leaflet of the plasma membrane, they enter the cell through the endocytic pathway. Alpha synuclein is then capable of inducing rupture of these internalized vesicles. Through the use of a vesicle rupture assay system involving stable expression of the fusion protein mCherry-Galectin3 (ChGal3), we can measure punctate re-localization to the Galectin 3 binding site on the inner leaflet of vesicles as vesicle rupture events. Through the colocalization analysis of these labeled rupture events and immunofluorescently labeled endocytic vesicle subtypes, our previous studies provide evidence that the vast majority of endocytic vesicle rupture induced by alpha synuclein occurs preferentially in lysosomes. We now aim to corroborate these findings by employing the same rupture assay system in conjunction with live-cell lysosomal markers. The same SH-SY5Y human neuroblastoma cells that have been transduced to stably express a fusion ChGal3 protein were treated with labeled alpha synuclein aggregates in addition to live-cell lysosomal dyes: either Lysotracker or Lysosensor. After imaging by deconvolution fluorescence microscopy and subsequent triple colocalization analysis, endocytic vesicle rupture and trafficking patterns have become apparent through the presence of various vesicle populations. Most notably and unexpectedly, there is a significant population of vesicles that undergo alpha synuclein induced rupture/permeabilization, but maintain luminal lysosomal markers. This suggests that some lysosomes can maintain or recover function after rupture. Further studies aim to confirm that these post-rupture lysosomes are functional by investigating the localization of lysosomal hydrolytic enzymes, namely Cathepsin B, before and after rupture. We expect that after lysosomal rupture occurs, Cathepsin B will be released into the cytosol to activate a known apoptotic pathway. These studies may allow for the identification of new therapeutic targets for the prevention of alpha synuclein induced cell death and resulting PD progression.

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Combining immunotherapy with a Cytomegalovirus-based vaccine for the treatment of murine melanoma

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Background/Hypothesis: With the recent clinical successes of immune checkpoint blockade, there is renewed interest in developing innovative strategies to direct host immune cells to fight cancer. Cytomegalovirus is a prevalent herpes infection that generates a unique CD8+ T cell response, characterized by a continuous accumulation of functional, activated cells over the lifetime of the host. Using a mouse model of melanoma, our group has recently shown that a Cytomegalovirus-based vaccine (MCMVgp100KGP) generating a lifelong CD8+ T cell response to the melanoma antigen, gp100, delays B16F10 tumor growth. However, mice eventually succumb to disease. To further improve this vaccine response, this study aimed to combine a MCMVgp100KGP with immune checkpoint blockade and adoptive cell transfer to treat a mouse model of B16 melanoma. **Methods:** C57BL/6 mice were inoculated with 105 B16F10 melanoma cells via intradermal injection. Three days later, mice received 105 MCMVgp100KGP or PBS via intraperitoneal injection. Tumor growth was measured every 2-3 days using calipers. Tumor Infiltrating Lymphocytes (TILs) were isolated following enzymatic digestion and analyzed by flow cytometry. For checkpoint blockade experiments, mice received 100µg αCTLA-4, 250µg αPD-1, or isotype control. For adoptive transfer, mice received an intravenous injection of naïve (PMEL) splenocytes from mice expressing a transgenic T cell receptor recognizing gp100, followed by vaccination. **Results:** After vaccination with MCMVgp100KGP, CD8+ TILs were present in higher numbers and expressed higher levels of the T cell activation marker CD11a ($p<0.05$; n=5-6). However, vaccination status did not influence expression of the inhibitory receptors CTLA-4 or PD-1 on CD8+ TILs (n=5-6), nor did combination

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of vaccination with CTLA-4 or PD-1 blockade influence tumor growth in a therapeutic setting (n=8-10). As predicted, MCMVgp100KGP did stimulate PMEL cells following transfer, and did so more effectively than another vaccine based on Vesicular Stomatitis Virus ($p<0.05$; n=3). **Discussion/Conclusions:** This data suggests that immune checkpoint inhibitors do not improve antitumor responses generated by Cytomegalovirus-based vaccines. One potential explanation is that TILs produced by MCMVgp100KGP are functional but too few to induce complete tumor regression. Adoptive cell therapy is one method to create larger T cell responses against tumor antigen. This study has shown that MCMVgp100KGP can stimulate transferred PMEL cells better than other viral vaccine vectors. Experiments are currently underway examining if vaccination with MCMVgp100KGP promotes lifelong persistence of transferred cells and how this combination influences B16 tumor growth. With the ability to elicit lifelong immune responses, Cytomegalovirus-based vaccines, alone or in combination with other immunotherapies, have the potential to induce enduring antitumor immune responses.

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Disruption of DDR2-collagen interactions with a novel small molecule inhibitor blunts cancer metastasis

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Discoidin Domain Receptor 2 (DDR2) is a receptor tyrosine kinase that utilizes the extracellular matrix protein collagen as its ligand. Recently, DDR2 was shown to be critical in facilitating breast cancer metastasis. In tumor cells, DDR2 expression (absent in normal breast epithelium) is induced during Epithelial Mesenchymal transition (EMT), and serves to stabilize protein levels of the EMT inducing transcription factor, Snail1. DDR2 expression is present in a majority of human invasive ductal breast carcinomas, and expression is localized to the tumor-stroma boundary. In these tumors DDR2 is acting in a positive feedback manner to maintain Snail1 levels and activity at the leading, invasive edge of the tumors, where cells have undergone EMT and come into contact with extracellular matrix (ECM) collagen I. This allows continued invasion through the ECM and contributes to breast cancer metastasis. Interestingly, in genetic models of breast cancer in mice, selectively eliminating DDR2 in either the tumor (MMTV-PyMT; DDR2 fl/fl; K14-Cre) or the fibroblasts within the stroma (MMTV-PyMT; DDR2 fl/fl; FSP-Cre) leads to a dramatic inhibition of tumor metastasis. This indicates that in addition to maintaining EMT in the tumor cells, DDR2 functions within the stromal compartment to facilitate metastasis. As such, DDR2 is a novel RTK target for the treatment of breast cancer metastasis, and we have developed potent and selective small molecule inhibitors of DDR2 that act at nanomolar cellular potency to block DDR2 binding to collagen. Unlike traditional tyrosine kinase inhibitors (TKIs) that target the intracellular kinase domain, the inhibitors that we have developed act on the extracellular domain of DDR2. These compounds provide a means of blunting breast cancer metastasis by targeting both the tumor and stromal cells, thereby disrupting DDR2 signaling in both compartments. Furthermore, using mouse models of late stage breast tumor metastasis, we have shown that these inhibitors reduce metastatic burden in the lungs of mice, to a level comparable of that of genetic knockdown of DDR2. Together these data support further investigation of this novel class of DDR2 inhibitors as anti-metastasis agents, potentially for use in combination with standard of care therapy to halt cancer progression and prevent relapse. Further, it will be important to study the role of these inhibitors in other cancers where DDR2 expression has been shown to promote metastasis, including, but not limited to, ovarian, head and neck, and nonsmall cell lung carcinoma.

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Aberrant R-loop processing in Juvenile Amyotrophic Lateral Sclerosis

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The mechanisms underlying motor neuron disease are still largely unknown, but increasing evidence shows that dysregulation of RNA processing plays a key role. Juvenile amyotrophic lateral sclerosis (ALS4) is a degenerative disease caused by autosomal dominant mutations in the RNA-DNA helicase, senataxin. The disease is characterized by slowly progressive weakness, with signs of both upper and lower motor neuron involvement. Senataxin resolves RNA/DNA hybrids (R-loops), which form during transcription as nascent transcripts hybridize with the unwound DNA duplex. These R-loops have been associated with alterations in RNA processing, genomic instability, and trinucleotide repeat sequence expansion. We seek to understand alterations in R-loops in ALS4, and how they relate to neurodegeneration. To address this question we have taken a two-pronged approach combining clinical and basic studies. We collected detailed phenotypical information from 12 individuals with ALS4, including MRI volumetric imaging of the thigh and brain. We sequenced the DNA and RNA samples from skin fibroblasts, lymphoblastoid cell lines, white blood cells, induced pluripotent stem cell (iPSC) lines, and differentiated FACS-sorted motor neurons from patients and controls. Results confirmed that senataxin is expressed in iPSC-derived motor neurons, other nervous tissues, and in all other tissues. By DNA-RNA immunoprecipitation with S9.6 antibody, we found a reduction in the abundance of R-loops in patient cells, consistent with senataxin's ability to resolve R-loops and likely gain of function of the ALS4 mutation. These observations suggest that alterations in RNA processing, specifically R-loop resolution, and the subsequent effects on gene expression, may adversely affect motor neurons in ALS4. Results from RNA-sequencing of

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fibroblasts shows differential expression of many TGF-b signaling genes including AKT3, SOS2, TGFbI, RASAL2, SMAD7, and SMURF1. Similarly, RNA-sequencing data from iPS-derived motor neurons also identified disruption of the TGF-b pathway including differential expression of FSTL3, CDKN2B, AHR, SYP, TGFbI, and NGF. In this presentation, I will describe the changes in R-loop profiles and alternation in gene expression in ALS4. Our investigations offer insight by providing a connection between R-loop and the biology of the disease, which may provide new targets for therapeutic development.

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MsrA protects against NF-κB-dependent cerebral ischemia-reperfusion injury

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Current treatment for acute ischemic stroke is designed to quickly restore blood flow through direct endovascular recanalization or the use of thrombolytic therapy. Paradoxically, cerebral vessel recanalization can cause further damage to brain tissue via reperfusion injury initiated by reactive oxygen species (ROS); however, little is known about the molecular mechanisms connecting elevated neurovascular ROS with ischemic stroke. We tested the hypothesis that protein methionine oxidation, a ROS-dependent post-translational modification, potentiates NF-κB activation and contributes to cerebral ischemia/reperfusion injury. First, we found that overexpression of MsrA, an antioxidant enzyme that reverses protein methionine oxidation, attenuated H2O2-augmented NF-κB activation in cultured human endothelial cells. To determine if NF-κB activity is regulated by protein methionine oxidation *in vivo*, MsrA-deficient (MsrA-/-) mice were crossbred with NF-κB-reporter (HLL) mice. MSRA-deficiency resulted in tissue specific increases in NF-κB activity in the lung and arteries of TNF-α-stimulated mice. Susceptibility to cerebral ischemia-reperfusion injury was assessed using a transient middle cerebral artery occlusion (tMCAO) model. Compared with wild-type mice, MsrA-/- mice had significantly larger infarct volumes, increased neutrophil infiltration, increased NF-κB activation, and more severe neurological impairment following tMCAO. Inhibition of the NF-κB pathway with a NEMO-binding domain (NBD) peptide prior to cerebral ischemia eliminated this phenotype. MsrA-/- mice also exhibited enhanced leukocyte rolling on mesenteric venules and upregulation of E-selectin, an endothelial NF-κB-dependent adhesion molecule known to contribute to neurovascular inflammation in ischemic stroke, suggesting that MsrA protects against post-ischemic inflammation by dampening NF-κB-mediated neutrophil-endothelial interactions. Finally, bone marrow transplantation experiments demonstrated that the neuroprotective effect is mediated by MsrA expressed in non-hematopoietic cells. We conclude that endogenous MsrA in the non-hematopoietic cell compartment protects against NF-κB-dependent neurovascular inflammation in acute ischemic stroke. These findings indicate that ischemic stroke is a redox-regulated process that can be modified by reversible methionine oxidation.

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Migration and Hypertension: A Meta-Analysis

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Background: Although previous work has attempted to generate evidence regarding the effects of migration on health, there are significant gaps in the knowledge regarding the effects of migration on hypertension. In this study, we examined the role of migration as a possible risk factor for hypertension by conducting a systemic review of the literature and a meta-analysis. **Methods:** Using PubMed searches and bibliographic reviews of published studies on human subjects, we identified those that reported the multivariate adjusted association between migration and hypertension or blood pressure as a primary or secondary outcome. The primary search generated 111 potentially relevant articles, of which 32 met the inclusion criteria. The strength of the association between migration status and hypertension was estimated by calculating crude and multivariate-adjusted pooled odds ratios (OR) using fixed- and random-effects models, along with their corresponding 95% confidence intervals (CI). Separate meta-analyses were carried out for the migrant as compared to the host population and to the origin population for both country-to-country and rural-to-urban migration studies. Data were analyzed with Stata 14.0 (Stata Corp, College Station, Tex). **Results:** Using random-effects models, we found that the overall OR comparing migrants to non-migrants (origin and host populations) was 1.04 (CI: 0.97, 1.10; p=0.27). In country-to-country migration studies, the prevalence of hypertension in migrants was similar to that in the host population (OR: 0.99, CI: 0.92, 1.06) but was 37% higher than that in the origin population (OR: 1.37, CI: 1.06, 1.77; p=0.01). In rural-to-urban migration studies the prevalence of hypertension in migrants was slightly, but not significantly, higher than in the host population (OR: 1.13, CI: 0.90, 1.42) and similar to that in the origin population (OR: 1.05, CI: 0.91, 1.21). However, there was considerable heterogeneity across studies (I2 values > 80.6%, p<0.0001). A meta-regression analysis including self-reported hypertension, publication year, and migration destination as covariates showed that migration to Europe was the only significant source of heterogeneity. **Conclusion:** Although there was some evidence that migrants in country-to-country studies had greater odds of hypertension than their origin populations, the interpretation of this finding is compounded by the large heterogeneity across studies. Differences in study design are the most likely source of heterogeneity. Furthermore, it is unclear whether migrants are comparable to their host and origin population. Future studies on the effect of migration on health should take into account these methodological issues.

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Exome Sequencing to Identify Novel Genes for Isolated Gonadot

WITHDRAWN

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Isolated gonadotropin deficiency (IGD) is a rare disorder of pubertal timing characterized by failure of GnRH secretion or action, which results in failure to enter or progress through puberty. While over thirty genes are known to cause the disorder, mutations in these genes collectively account for only one third of disease cases. Thus, there remains much to be discovered about the genetics and corresponding biology of IGD. To find novel genes for IGD, we performed exome sequencing in a cohort of 222 unrelated IGD cases of European ancestry. We then developed a novel gene-based burden testing approach by comparing the case data against publicly available exome sequencing in ~33,000 non-Finnish European individuals from the Exome Aggregation Consortium (ExAC). Importantly, we demonstrated that our approach is controlled for type I error. As positive controls, our strategy identifies known IGD genes (such as KAL1, FGFR1, and PROKR2) with strong statistical signal (p< 2.5 x 10-6). We also demonstrate how the strategy can be refined to increase the genetic signal and accommodate various genetic architectures. Our initial results identify other genes not previously implicated in IGD that bear suggestive statistical signals for association. Some of these candidate genes nominated by our analyses are being carried forward into functional studies and in expanded genetic analyses in even larger cohorts and in individuals of non-European ancestries. In conclusion, our studies apply a novel approach to finding disease genes for IGD, leveraging large-scale genomic sequencing and comparing with publicly available sequencing in a resource-efficient manner.

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Adult cortical motoneurons demonstrate robust growth 6 months after conditional PTEN deletion

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Previous studies have reported neuronal hypertrophy and cortical enlargement following deletion of the phosphatase and tensin homolog on chromosome 10 gene (PTEN) in developing mice and in newborn neurons in adult mice. Here, we show that deletion of PTEN in adult cortical motoneurons in the sensorimotor cortex triggers remarkable neuronal enlargement. Using an approach that has previously been shown to promote axon regeneration and functional recovery following spinal cord injury, AAV-Cre was injected unilaterally into the sensorimotor cortex of adult (8+ weeks old) mice with a lox-P flanked exon 5 of the PTEN gene. These mice also have a lox-P flanked STOP cassette that regulates expression of a red fluorescent Cre-reporter gene (tdTomato). Control mice have the Cre-reporter gene and a wild-type PTEN gene, and they were given identical intracortical injections of AAV-Cre. Mice were allowed to survive for 3-12 months after AAV injections. All mice also received bilateral fluorogold injections at cervical level 5 of the spinal cord to retrogradely label the cortical motoneurons that give rise to the corticospinal tract. Brain sections were immunostained for PTEN to identify the region of PTEN deletion and for phosphorylated ribosomal protein S6 (pS6), which is a marker for activation of the growth-promoting mammalian target of rapamycin (mTOR) pathway. In unstained sections the area of PTEN deletion was identified by the presence of tdTomato positive cells, typically in a region about 1mm in diameter. Immunostaining for pS6 revealed higher levels of immunostaining of neuronal cell bodies and processes within the region of PTEN deletion even up to a year after PTEN deletion, suggesting maintained mTOR pathway activation. Quantitative assessments of cortical motoneuron cell body size revealed that retrogradely labeled PTEN-deleted neurons were not significantly larger than control neurons 3 months after inducing PTEN deletion (Two-way ANOVA, p=0.1980). Performing identical analyses of PTEN-deleted cortical motoneurons 6 months after deletion revealed the development of significant cell body hypertrophy compared to control neurons (Two-way ANOVA, p<0.0001). Our results indicate that deletion of PTEN in adult neurons re-initiates an ability for robust neuronal growth that is normally restricted to the developmental state. These findings suggest novel strategies to prevent degenerative changes in adult neurons due to aging, traumatic injury, or neurodegenerative disease.

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The Molecular Profiling of Type 1 Diabetic Pancreatic Tissue using Imaging Mass Spectrometry

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Progress in understanding the pathologic changes in the human pancreas in Type 1 Diabetes (T1D) is limited by the relatively small number of human samples studied and because the application of some new analytical technologies is hindered by how these tissues are processed. For example, information about lipids or metabolites in T1D islets is largely absent from most analyses because classical histological techniques have limited ability to detect these molecular species and because tissue processing often removes lipids. To better understand the molecular changes in the T1D pancreas, we used imaging mass spectrometry (IMS), which offers both molecular specificity and spatial information for multiplexed, regiospecific measurements of proteins, lipids and metabolites on intact tissue, to examine the exocrine and endocrine compartments in normal and T1D pancreata. We examined pancreatic tissue from normal (n=2; 19-month-old female and 20-year-old male) and T1D individuals (n=2; 12-year-old female, 3 years T1D and 20-year-

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old male, 7 years T1D). Donor tissue was flash frozen, and 10 µm-thick sections were mounted onto indium tin oxide slides that were subsequently treated with a MALDI matrix. The tissue section adjacent to that prepared for IMS was analyzed by immunofluorescence for islet hormones, and then islets were co-registered with those on the IMS slide during analysis. IMS experiments, performed on a 15T FTICR, produced spectra specific for protein and lipid species, which were further identified using tandem mass spectrometry (MS/MS). IMS readily detected protein and lipid species in normal pancreatic islets that were distinct from those in exocrine tissue. We identified protein signals unique to islet cell types, with protein signal m/z 3031 concentrated in alpha cells and a m/z 3961 signal associated with beta cells, as well as differential localization of phosphatidylcholines (PC) lipids specific to the endocrine [PC(16:0/18:1), PC(18:1/18:2), PC(18:0/20:4)] versus exocrine [PC(16:0/18:2)] compartments. T1D islets had decreased expression of arachidonic acid-containing phospholipids (20:4), decreased expression of sulfatides (sulfagalactosyl ceramides), and differences in plasma membrane PC lipid fatty acid tail composition. Pancreatic islet protein and lipid species, as detected by IMS, are differentially expressed in endocrine and exocrine compartments, and T1D islets have a distinct lipid distribution.

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Non-canonical activating effects of alpha2a adrenergic receptor agonism in the bed nucleus of the stria terminalis

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Drug addiction is a major health concern. Patients often go untreated or undertreated, leading to relapse. Stress is a major antecedent of relapse. During protracted abstinence, elevated levels of brain norepinephrine (NE) engage maladaptive stress circuitry to promote reinstatement. Agonism at α2-adrenergic receptors (α2-ARs) can dampen this elevated NE tone. Clonidine and guanfacine are α2-agonists with positive preclinical results dampening stress-induced reinstatement of drug seeking behavior. However, ultimate rates of relapse in humans are unchanged by this treatment. We hypothesize that this is due to competition among the effects of α2-AR agonism beyond its commonly cited role as an inhibitory autoreceptor on NE terminals. We aim to investigate these effects in the bed nucleus of the stria terminalis (BNST), a component of the extended amygdala implicated in the integration of stress and reward in the dependent brain. In rodent models, direct administration of α2 agonists reduces stress-induced reinstatement behaviors. In the BNST, α2-AR agonism can inhibit release of both norepinephrine and glutamate from presynaptic terminals, with the latter being a specific effect on afferents from the parabrachial nucleus (PBN). Recently, we have found that α2-AR agonism can produce enhancement of excitability in BNST. However, the mechanism underlying these effects, as well as the specific identification of the cells activated, are critical unknowns. We aim to determine the mechanism underlying α2-AR agonism-induced enhancement of glutamatergic transmission in BNST neurons and its relevance to circuit activity, and to begin to determine the impact of this regulation by understanding the population of cells regulated. We hypothesize that activation of postsynaptic α2A-ARs enhances excitatory responses in a population of BNST neurons through inhibition of HCN channels. To test this hypothesis, we combine electrophysiological studies aimed at uncovering the mechanism of guanfacine activating effects within the BNST with anatomical studies aimed at identifying the guanfacine-activated population of BNST neurons. Here, we show that guanfacine-activated BNST neurons show a high prevalence of the hyperpolarization-activated current Ih, suggesting a role for HCN channels in the activity-enhancing effects of α2A-AR agonism. In addition, HCN channel inhibition mimics guanfacine effects on postsynaptic field potential responses in the BNST from the Thy1-COP4 transgenic mouse line. Complementary anatomical data shows that postsynaptic α2A-AR mRNA expressing BNST neurons are largely distinct from HCN1 subunit-expressing neurons, suggesting the HCN2 subunit mediates guanfacine enhancing effects on BNST neuronal activity. Through these experiments, we hope to gain a better understanding of non-canonical effects of α2-AR agonism in the BNST and its behavioral and circuit relevance.

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Pathobiont colonization resistance by type VI secretion

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The importance of pathobionts in the pathogenesis of inflammatory bowel disease (IBD) and colonic malignancy is increasingly recognized. One such organism, Enterotoxigenic *Bacteroides fragilis* (ETBF), a subspecies of *B. fragilis,* releases *B. fragilis* toxin (BFT), a zinc-dependent metalloprotease that causes a pro-inflammatory injury of the intestinal epithelium. ETBF is present in stool samples from individuals with acute exacerbations of IBD, and infection of experimental animals with ETBF produces an IBD-like colitis. Further, ETBF has been implicated in the pathogenesis of colonic malignancy. ETBF acquisition accelerates tumor formation in susceptible mice, and was demonstrated to be associated with 100% of late-stage human colon cancer lesions. In contrast to ETBF, non-toxinogenic *B. fragilis* (NTBF) is an intestinal symbiont that provides protection from inflammatory disease. Humans exhibit variable patterns of colonization with NTBF and ETBF, harboring either of the subspecies independently or both simultaneously. ETBF colonizes 5-20% of asymptomatic humans, suggesting that these individuals may incur an underappreciated long-term health risk from chronic colonization. One potential strategy to remove this risk is targeted colonization resistance for pathobionts. However, the mechanisms that underlie human susceptibility or resistance to ETBF acquisition remain undefined. As B. fragilis populates the human microbiota in the first weeks

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of life, we hypothesize that colonization of NTBF may either restrict or permit ETBF acquisition, dependent upon modular genetic determinants of competition. To test this, we established a mouse model for sequential infection of B. fragilis strains including three NTBF and two ETBF human isolates. Exploration of all pair-wise primary and secondary colonization combinations revealed that restriction or tolerance of secondary colonization is a principal property of competition. It was recently discovered that B. fragilis encodes a type VI secretion system (T6SS): an effector delivery system, used for contact-dependent killing of non-self organisms. While it has been theorized that the microbiota uses the T6SS to prevent colonization of pathogens, this has never been demonstrated *in vivo*. We found that mutation of the T6SS in a restrictive strain of NTBF relieves the colonization resistance toward ETBF, allowing for enhanced colonization. We also demonstrate that NTBF uses the T6SS to decease the bacterial load of ETBF during co-infection. These two models reveal that the T6SS of NTBF reduces colonization of ETBF, thereby decreasing host exposure to toxin. These studies suggest that a molecular understanding of bacterial competition can be utilized to preclude toxin-mediated disease through restriction of pathobiont infection, potentially defining a novel disease-modifying therapeutic strategy.

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Detection of peptidoglycan from the gut microbiota governs the lifespan of circulating phagocytes at homeostasis

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Maintenance of myeloid cell homeostasis requires continuous turnover of phagocytes from the bloodstream, yet whether environmental signals influence phagocyte longevity in the absence of inflammation remains unknown. Here, we show that the gut microbiota regulates the steady-state cellular lifespan of neutrophils and inflammatory monocytes, the two most abundant circulating myeloid cells and key contributors to inflammatory responses. Treatment of mice with broad-spectrum antibiotics, or with the gut-restricted aminoglycoside neomycin alone, accelerated phagocyte turnover and increased the rates of their spontaneous apoptosis. Metagenomic analyses revealed that neomycin altered the abundance of intestinal bacteria bearing γ-D-glutamyl-meso-diaminopimelic acid (iE-DAP), a ligand for the intracellular peptidoglycan sensor Nod1. Accordingly, signaling through Nod1 was both necessary and sufficient to mediate the stimulatory influence of the flora on myeloid cell longevity. Transmission of Nod1-dependent signals to circulating phagocytes required the liberation and action of the pro-inflammatory cytokine interleukin 17A (IL-17A). Together, these results define a mechanism through which intestinal microbes govern a central component of myeloid homeostasis and suggest perturbations of commensal communities can influence steady-state regulation of cell fate.

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Spontaneous immune dysregulation in the skin in the absence of Wiskott-Aldrich Syndrome protein

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Wiskott-Aldrich Syndrome (WAS) is a severe X-linked immunodeficiency caused by deficiency of Wiskott-Aldrich Syndrome protein (WASp) in hematopoietic cells. WASp is a key regulator of actin polymerization and is critically important for leukocyte migration, polarization, and effector function. WAS patients exhibit a range of pathologies associated with heightened type 2 immune responses, including elevated serum IgE, treatment-refractory atopic dermatitis (AD), and colitis, but they also suffer from recurrent pyogenic infections, suggesting a defect in type 1 responses. Why WAS patients develop type 2 pathologies and autoimmunity despite significant functional defects in leukocytes is still unclear. We have previously demonstrated that WASp-deficient CD4+ T cells have defects in type 2 cytokine secretion while innate effectors, including basophils and γδ T cells, have intact production of IL-4. Using a mouse model of WASp deficiency, we seek to define the relative contributions of innate and adaptive lymphoid populations to the development and maintenance of type 2 pathologies. WAS patients develop severe AD, but overt skin pathology has not previously been described in WASp-deficient mice. We observed local (skin) and systemic increases in type 2 innate effectors, including basophils, eosinophils, and group 2 innate lymphoid cells, which could contribute to type 2 pathology predisposition. The changes in skin immune populations predate serum IgE elevation, suggesting that local alterations at barrier surfaces may drive systemic shifts in immunity. Unexpectedly, we also observed a homeostatic increase in the number of γδ T and RORγt+ cells, both systemically and locally in the skin. This perturbed baseline suggests coexisting type 2 and type 17 inflammation, which has been described in some chronic cases of asthma and AD. Paralleling our findings with type 2 cytokine release, we observed a defect in CD4+ T cell secretion of IL-17 despite robust production of IL-17 by γδ T cells, highlighting a differential role for WASp in function of type 17 innate and adaptive immune effectors. We hypothesize that altered immune barrier function may drive the pathologies observed in WAS and may contribute to lack of patient response to standard AD treatment regimens. A better understanding of how WASp deficiency impacts type 2 and 17 responses *in vivo* may provide insight for alternative interventions for WAS patients and new therapies for the control of concomitant type 2/type 17 pathologies in non-WAS settings.

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Candida albicans and the murine immune system interact during gastrointestinal colonization

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The opportunistic fungal pathogen Candida albicans commonly and asymptomatically colonizes the human gastrointestinal (GI) tract. Immunocompromised individuals, however, can develop life-threatening candidemia, the main source of which is C. albicans colonizing the individual's own GI tract. Our goal was to examine the interaction between a host's immune system and the Candida colonizing its GI tract. When we examined the expression of cytokines in murine stomach tissue, we found that IL-22 transcripts, similar to previous reports, and also IL-17 transcripts were over 100-fold higher in C. albicans-colonized stomachs, compared to uncolonized controls. In contrast, IL-10 and IFNγ transcript levels did not significantly differ between colonized and uncolonized mice. When we examined the gene expression of colonizing C. albicans cells, we found that several Candida genes' transcripts were significantly more abundant in cells colonizing immunocompetent mice that had been previously exposed to Candida, compared to cells colonizing immunocompromised mice that lacked functional T-cells. Consistent with previous reports, these genes included EFG1, a transcription factor important for the ability of C. albicans to colonize the GI tract and to undergo filamentation, a virulence trait. These differentially-expressed genes also encoded histone modifying proteins involved in regulation of EFG1 expression, and components of the Efg1-mediated filamentation pathway. Finally, while immunocompetent mice typically develop a Candida-restrictive colonization phenotype after their initial exposure to Candida, we found that this phenotype was delayed in mice treated with an anti-CD4 antibody. In summary, in a murine model of GI tract colonization, Candida colonization elevates specific cytokine transcript levels in the GI tract, multiple EFG1-related Candida transcripts are differentially regulated in hosts of different immune status, and maintenance of a host's Candida-restrictive phenotype is dependent upon CD4+ cells. Therefore, the host immune system and the colonizing Candida interact even before the development of overt disease, a relationship which should be further examined in order to inform our goal of preventing the development of candidemia.

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A predictive analytics platform leveraging large-scale claims data for estimating fall risk in people aged 65 years and older

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In 2014 alone, more than 650,000 people aged 65 years or older in the United States suffered an accidental fall, resulting in over 24,000 deaths. Alarmingly, the rate of these deaths has nearly doubled (30 to 57 per 100,000 per year) from 2000 to 2013. Here, we develop a first-of-its-kind predictive analytics platform for estimating fall risk in this older population, based upon claims data from a nationwide insurer with over 30 million members. The technology extracts predictive features from data elements commonly found in insurance claims and then employs machine learning methods to calculate an individualized risk of falling within the next two years. Specifically, the system queries for demographics (age and sex), prescriptions (National Drug Codes), as well as diagnoses (ICD-9 codes). Mapping techniques, including a novel method to classify National Drug Codes into ATC categories, group the codes. The process results in 281 predictive features which the platform uses to fit a logistic regression model, with lasso for feature selection. The predictive model stratified the 116,452 members in the held out test data into one of 20 risk levels (approximately 5,820 members per level). Those in the higher risk level had a 35% risk of falling in the next two years and their risk was 16.3 times more than those in the lowest risk level (2%). Across the 20 levels, the error between the estimated and actual probability assigned to a given risk level was within 4.6% on average. These results demonstrate the method's ability to effectively risk stratify the population. The stark difference between the lowest and highest risk levels, coupled with the fact that the prediction is made a year in advance on average, suggest the technology can provide timely, actionable, personalized assessments for each patient. Since the data-driven approach draws from typical insurance claims data, it can be widely applicable. Furthermore, future enhancements such as use of nonlinear predictive models (e.g., random forests) or addition of entries common to electronic medical records (e.g., laboratory test values) promise to yield even higher gains in accurately estimating risk. For these reasons, the research is thought to usefully contribute to the larger effort of reducing and mitigating the widespread occurrence of accidental falls in those 65 years and older.

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TKA Polyethylene Debris Migrates Along the Cement-Bone Interface

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Introduction: Aseptic loosening is the primary cause of premature failure of cemented Total Knee Arthroplasty (TKAs). Aseptic loosening is largely attributed to inflammation caused by polyethylene (PE) wear debris generated from the articulating surface, evidenced by debris found in peri-prosthetic tissue during revision procedures (after failure). As the role of PE debris in successful TKAs has not been determined, we asked if PE debris accumulates at the cement-bone interface with years in service, and if PE debris is locally associated with bone resorption. **Methods:** Six fresh, non-revised, postmortem retrieved TKAs were obtained en bloc from the SUNY Upstate Anatomical Gift Program. For each, the metal tibial component was removed, leaving the cement-bone interface intact. Frozen (-80°C) tibias were sectioned in 10mm intervals in the

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sagittal plane starting from the midline. The proximal 15mm containing the cement-bone interface were fixed in 10% neutral buffered formalin, dehydrated in graded alcohols, and embedded in Spurr's Resin. Specimens were sectioned in the coronal plane, then ground and polished to <0.5mm. Polarized light microscopy was used to quantify birefringement PE wear particles/1mm2 at the interface (n >100/specimen), and deep to the interface (n >100/specimen). Two lab-prepared specimens without prior joint replacement were used as negative controls **Results:** Loss of trabecular bone Interdigitation Depth (LossID) increased with years in service in a logarithmic fashion. The average PE burden/mm2 (SE) for specimens with time in service (2.25-20 years) ranged from 12.38 (1.26) to 22.71 (2.96), and increased with years in service. LossID was not related to Average PE Burden in specimens with years in service. Hood Wear Score (wear on the PE insert) correlated well with the average PE burden, indicating consistency of polarized light microscopy analysis. LossID was not correlated to # PE per region of interest. There was no consistent regional distribution of accumulated PE debris. **Discussion:** We documented that the PE debris generated at the articulating surface of the TKA can migrate along the cement-bone interface of well-fixed tibial components. However, the amount of PE debris was unrelated to the amount of local bone loss, suggesting important, alternative causes of clinical aseptic loosening apart from PE debris. **Significance:** The absence of local resorption (LossID) in the presence of PE demonstrates that trabecular bone resorption may occur independently of PE debris at the interface. As the loss of the interlocking trabeculae leads to subsequent loosening of the implant and eventual revision, identifying the alternative causes of aseptic loosening are essential to preventing premature implant failure.

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Drosophila Myc regulates circadian locomotor behavior and metabolism in flies

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Drosophila Myc (called dMyc) is a transcriptional factor that is highly conserved with functional properties similar to mammalian Myc, including regulation of cell growth, development, and metabolism. We recently reported that mammalian Myc disrupts molecular clock and clock-regulated glucose metabolism in cultured cancer cells through upregulating Rev-erbα, the repressor of major clock regulator Bmal1 (Altman and Hsieh et al. Cell Metabolism Sep 2015). However, the role of dMyc in Drosophila molecular clock and metabolism is not known. Here, we demonstrate that both gain-of-function and loss-of-function of dMyc can disturb circadian locomotor behavior in flies. Using the Drosophila Activity Monitoring (DAM) system, we show that overexpression of dMyc specific in Pigment Dispersing Factor (PDF) and Cryptochrome (Cry) expressing cells result in higher percentage of arrhythmic flies. In these flies, dMyc overexpression also induces several clock-controlled genes, such as Cyc, Tim, Cry and Cwo. Morphological analysis of neurons required for circadian locomotor behavior in the dMyc-overexpressing flies showed decreased PDF staining in the dorsal projection while the cell bodies remain unchanged. Preliminary metabolic profiling of the heads from these flies using GC-MS revealed that high dMyc leads to significant consumption of valine and leucine, accompanied with accumulation of urea. Whether neuron development or metabolic alteration results from high dMyc is the underlying mechanism of arrhythmicity requires further investigation. Interestingly, adult dMyc mutant flies also display higher percentage of arrhythmic circadian activity, which can be rescued by mutation of dMnt, a known suppressor of dMyc activity. In contrast to dMyc overexpression, loss of dMyc does not alter the expression and oscillation of clock-controlled genes. However, PDF projection is dramatically diminished in dMyc mutant flies and can be rescued by dMnt mutation, suggesting endogenous dMyc is essential for circadian output, particularly PDF. Our results demonstrate a novel role of dMyc in modulating Drosophila circadian locomotor behavior potentially through directly regulating components of the core molecular clock as well as indirect effect on clock neuron development, clock outputs and metabolism. We thank the following funding sources: NIH R01CA051497, R01CA57341, LLS 636311

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TGFβ signaling in the C. elegans germline regulates prostaglandin metabolism important for sperm targeting

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Fertilization is a critical event that maintains species diversity and population survival. Successful fertilization requires that the sperm locate and fuse with the oocyte. The signaling mechanisms involved in this process have been well characterized in marine species, but less is known about the process that guide sperm to oocytes in internal fertilizing organisms. C. elegans' clear hypodermis allows for the direct visualization of sperm motility within the oviduct. Previous studies using C. elegans have identified a class of F-series prostaglandins (PGFs) that are critical for sperm guidance toward oocytes. C. elegans lack classical cyclooxygenase (Cox) enzymes, and oocytes synthesize specific PGFs via a novel, unidentified mechanism. Similar PGFs have been found in Cox knockout mice and human follicular fluid, suggesting related mechanisms exist in mammals. Previously, our lab has shown that DAF-7, a homologue of TGFβ, couples environmental cues to oocyte PGF synthesis, and that mutations in daf-1 type I TGFβ receptor causes sperm guidance defects that can be repressed by daf-3 co-SMAD loss. My unpublished data indicate that DAF-3 represses oocyte PGF synthesis in daf-1 mutants. DAF-3 activity is required in part in transcriptionally active oocyte precursors, suggesting that DAF-3 transcriptional targets modulate PGF metabolism. I am conducting RNA-seq, together with RNAi and lipidomic studies to identify critical DAF-3 targets and potential biochemical steps. These studies are providing insight

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into molecular mechanisms of fertilization, as well as a novel pathway(s) for PGF metabolism.

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Visualizing Mouse Egfr *in vivo* via Targeted Genome Editing with CRISPR/Cas9

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The epidermal growth factor receptor (EGFR) signaling pathway is involved in many different cellular functions. In addition, the EGFR is an important therapeutic target in many solid tumors. A major limitation in studying the Egfr in normal and disease states in the mouse is the lack of robust and reliable antibodies to visualize mouse Egfr. To overcome this limitation, we generated an Egfr reporter mouse. By CRISPR/Cas9 gene editing, a DNA cassette encoding Emerald GFP (EmGFP) was inserted between exon 28 of Egfr and the 3'UTR, leading to production of C-terminal-tagged Egfr-EmGFP fusion protein under the control of endogenous promoter. The reporter line, EgfrEmGFP, was confirmed by sequencing of full-length cDNA and detection of Egfr-EmGFP protein. Homozygous EgfrEmGFP mice were viable and fertile. We derived primary mesenchymal stem cells from homozygous EgfrEmGFP mice to test the signaling capability of Egfr-EmGFP. These cells were treated with recombinant mouse (rm)EGF (20ng/ml); there was robust activation of Egfr in five minutes as determined by p-Egfr (Y1173), followed shortly thereafter by a transient increase in p-Erk1/2 and p-Akt, thus recapitulating events following activation of endogenous Egfr. To investigate Egfr activity and dynamics *in vivo*, an intraperitoneal injection of rmEGF (one mg/gram body weight) was given to EgfrEmGFP mice and livers were harvested at different time points. Membranous GFP staining was observed at baseline in hepatocytes. Five minutes after rmEGF injection, hepatocytes exhibited increased membranous staining; at 30 minutes, there was decreased membranous staining and increased cytoplasmic staining. Egfr-EmGFP protein expression pattern returned to baseline at 24 hours. These data demonstrate the sensitivity of this reporter line for *in vivo* subcellular localization of Egfr. Various organs/tissues were collected to study the dynamics of Egfr-EmGFP expression. One interesting example was that, contrary to previous reports, GFP staining was confined to the crypt and villi were devoid of staining in the small intestine. The proximal colon showed GFP staining at the crypt base, whereas staining was throughout the crypt in the distal colon. This new Egfr reporter mouse line will be a valuable tool to monitor Egfr localization and dynamics in physiologic and pathologic states.

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Cdkn2a is an essential target of EZH2 during B and T cell development

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The histone methyltransferase EZH2 catalyzes the H3K27me3 histone modification, which is broadly associated with repressed and poised chromatin. EZH2 is frequently mutated in blood and epithelial tumors and increased EZH2 activity enhances tumorigenicity. With the introduction of EZH2 inhibitors, the normal functions of EZH2 must be elucidated. EZH2 is required for both B and T cell development, but its specific role in these lineages is not clear. Here we used an Il7racre to delete Ezh2 in all lymphocytes. We confirmed that EZH2 is required for B and T cell development, but found that it was dispensable during NK cell development. EZH2-deficient pro-B cells failed to expand *in vitro*, due to increased apoptosis and cell cycle inhibition. This was associated with upregulation of the cell cycle regulators, p16/INK4a and p19/ARF, which are encoded at the Cdkn2a locus and are known targets of EZH2 in other tissues. ARF prevents ubiquitin-mediated degradation of the tumor suppressor p53 leading to cell cycle defects and apoptosis. p53 was increased in EZH2-deficient pro-B cells and canonical p53 target genes were also increased. To determine whether Cdkn2a is an essential target of EZH2 during B and T cell development, we crossed EZH2-deficient mice to the Cdkn2a-/- background. Abrogation of Cdkn2a partially rescued B cell development and robustly restored T cell development in EZH2-deficient mice.

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GAB2 signaling in ovarian cancer

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We recently found that the gene encoding the scaffold protein GAB2 [growth factor receptor-bound protein 2 (GRB2)-associated binding protein 2] is amplified and overexpressed in a subset of primary high-grade serous ovarian cancers (HGSOCs), which represent the most aggressive subtype of ovarian cancer. GAB2 has been implicated in mediating signal transduction from receptor tyrosine kinases to various downstream pathways such as p85, SHP2, and CRKL, but the molecular and cellular mechanisms underlying its frequent amplification and overexpression in HGSOCs remain elusive. HGSOCs are often diagnosed at advanced stage with widely disseminated tumors in the peritoneal cavity. One characteristic feature of ovarian cancer cells is the ability to survive and embed into the lining of the peritoneal cavity, which serves as an 'outpost' for subsequent extensive metastatic invasion into vital organs. Integrin signaling has been shown to play important roles for each of these processes. We hypothesized that overexpression of GAB2 in ovarian cancer cells promotes metastatic cell invasion and tumor growth through enhancing integrin signaling. We showed that overexpression of GAB2 in MEK-transformed human

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fallopian tube secretory epithelial cells enhanced their ability to form metastatic tumors when these cells were implanted intraperitoneally into immunodeficient mice. Suppression of GAB2 in ovarian cancer cells with GAB2 amplification induces tumor growth arrest in immunodeficient mice. These results demonstrated that ovarian cancer cells harboring GAB2 amplification/overexpression might have developed strong dependence on oncogenic GAB2 for tumor growth. We obtained further evidence that overexpression of GAB2 in immortalized fallopian tube secretory epithelial cells and ovarian cancer cells promoted interaction between GAB2 and CRKL adapter proteins which have been implicated in integrin signaling. Suppression of GAB2 or CRKL using single guide RNA-mediated gene knockout technology in ovarian cancer cells with GAB2 amplification/overexpression disrupted formation of stress fibers and decreased number and size of focal adhesions. To further investigate the biological significance of the GAB2-CRKL interaction, we have generated a GAB2 mutant in which 6 putative tyrosine residues responsible for binding to CRKL were mutated to phenylalanine residues. We are currently assessing whether disruption of CRKL binding affects the ability of GAB2 to induce cell transformation and metastatic tumor growth in ovarian cancer cells. Therefore, these studies will establish CRKL as a downstream effector of GAB2 overexpression critical for ovarian cancer cell invasion and metastatic growth.

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Antibodies in the nervous system during latent HSV-1 infection

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Herpes simplex virus type-1 (HSV-1) is a neurotropic pathogen persisting in the majority of the human population. Despite its high prevalence, curative medicine and vaccines remain elusive. The inability of therapeutics to completely eradicate the virus and the high recurrence of disease are mainly due to capacity of HSV-1 to become latent in the trigeminal ganglion (TG). Although antibodies are important in combating acute HSV-1 infection, their role in controlling latent infection is poorly understood. While previous studies focus on the role of T cells in the TG, our data from a mouse model show that HSV-1 specific IgG is also persistent and enriched in latently infected TGs. Furthermore, local antibody-producing cells migrate into the TG shortly after the acute phase of infection. Therefore, our data are consistent with the idea that locally produced TG antibodies against HSV-1 may be heretofore unrecognized suppressors of viral reactivation. We are currently testing this hypothesis with B cell deficient mice. Our future work will elucidate the idea that persistent antibody may be important in both protection and disease in the nervous system.

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Dysregulation of tissue-reparative regulatory T cell function in infectious myositis

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While immune dysfunction is a central event in the pathogenesis of idiopathic inflammatory myopathies, the etiology of this immune dysfunction remains elusive. Recently, a growing body of evidence has demonstrated that in other tissues, acute and chronic infections may permanently alter tissue-specific immune processes. As tissue repair is an immune-mediated process, it is interesting to postulate that chronic infection may have long-lasting effects on the regulation of skeletal muscle repair, lending to the genesis of inflammatory myopathies. Skeletal muscle repair is heavily reliant on the temporally distinct transition from pro-inflammatory (M1-polarized) to pro-regenerative (M2-polarized) macrophage-mediated phases of repair. This process is coordinated by regulatory T cells (Tregs) and dysregulation of this transition (persistence of M1 populations) results in debilitating chronic injury and fibrosis. Here, we report that infection with Toxoplasma gondii alters the regulation of macrophage polarization in skeletal muscle through permanent disruption of the overall Treg compartment. Following infection, the skeletal muscle immune landscape is remodeled toward a type 1 inflammatory environment with dramatically decreased Treg frequencies that fail to fully recover up to 100-days post-infection. In this context, a significant M1 population persists in the skeletal muscle that is typically dominated by M2 macrophages at steady-state. Because Tregs are purported to exert a pro-regenerative role in part by promoting the transition from M1 to M2 macrophages, we asked whether an alterations in Treg populations during infection were responsible for the observed dysregulation of macrophage polarization. Surprisingly, systemic depletion of Tregs in infected mice increased muscle fiber regeneration through increased M2:M1 ratios without an increase in inflammatory cells, suggesting this proposed role of Tregs is altered during infection. Therefore, we hypothesized that in the context of an infection-induced type 1 inflammatory environment, Tregs instead promote M1-polarization. To test the effect of chronic interferon-gamma exposure on Treg function, we adoptively transferred induced Tregs with and without exposure to interferon-gamma *in vitro*. Adoptive transfer of Tregs exposed to interferon-gamma enhanced the M1 population in infected skeletal muscle, whereas transfer of non-polarized Tregs had similar proportions of M2:M1 as transfer controls. Collectively, our results suggest that chronic inflammation associated with T. gondii infection not only permanently impairs the ability of tissue resident Tregs to promote tissue regeneration but these Tregs actively participate in immunopathology of the skeletal muscle. As Treg-directed therapies gain traction, a deeper understanding of the consequences of chronic inflammation on Treg physiology and tissue-specific reparative programing will be a powerful asset in producing safe and efficacious therapies.

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Clinical Manifestation and Outcome in Patients with Takotsubo Cardiomyopathy Accompanied by Psychiatric Disorder

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Background: Takotsubo Cardiomyopathy (TTC) is a heart condition that presents similarly to myocardial infarction but shows no coronary lesions upon further investigation. The apical ballooning configuration seen on angiogram gives the disease its name, which comes from the Japanese word for octopus pot, "tako tsubo." TTC is frequently provoked by an emotional triggering event, such as the death of a loved one. Therefore, in many instances, mental health disorders can predispose individuals to developing TTC. This study sought to elucidate the differences in short and long term outcomes between TTC patients with and without psychiatric disorders. **Methods:** Data regarding patient demographics, in-hospital complications, corrected QT (QTc) interval, recurrence and mortality was collected from 1496 patients included in the International Takotsubo Registry (InterTAKRegistry), a compilation of the efforts of 26 centers in nine countries. The patients studied were split into two groups based on the diagnosis of psychiatric disorders, which include acute or chronic affective, anxiety or adjustment disorders. Those patients with a history of acute or chronic psychiatric disorders were included in the TTC-PSYCH cohort and were compared to a TTC cohort of patients who had no previous psychiatric history. Short term outcomes of the two cohorts were measured using in-hospital complications such as catecholamine administration, cardiogenic shock and death, while long term outcomes were measured using recurrence and mortality rates at a 10-year follow up. **Results:** TTC-PSYCH patients were younger (64.7 ± 13.5yrs vs. 67.0 ± 12.8yrs; P=0.003) and more likely to be female (91.6% vs. 88.5%; P=0.09). They also presented with more emotional triggering factors than the TTC cohort (36.5% vs. 22.7%). The QTc interval was found to be more prolonged in TTC-PSYCH patients (461.9 ± 48.9ms vs. 454.6 ± 48.8ms; P=0.042), even though a subanalysis revealed no significant difference in QTc duration between females and males (P=0.85). Hypothyroidism (18.5% vs. 11.3%; P=0.001) and migraine (9.8% vs. 4.0%; P<0.001) were more prevalent among the TTC-PSYCH patients. Although we found short term outcome (P=0.65) to be comparable, TTC-PSYCH patients had a higher 10-year recurrence rate (P=0.002) but no significant difference in mortality rate when compared to TTC (P=0.70). **Conclusion:** TTC patients with psychiatric disorders have distinctive clinical features and higher long term recurrence rates. Therefore, we suggest that these patients should be closely monitored via routine follow-up visits to improve long term outcome

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VEGF regulates local [illegible] activity in the eye [illegible]

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Outer retinal and renal glomerular functions rely on specialized vasculature maintained by tightly controlled levels of VEGF produced by neighboring epithelial cells; the retinal pigment epithelium (RPE) and podocytes respectively. Changes in local VEGF production leads to vasculature pathology. Increased RPE-derived VEGF is thought to cause neovascularization in wet age-related macular degeneration (ARMD) while depletion causes choriocapillaris degeneration and atrophic changes in a mouse model. Reduced podocyte-derived VEGF causes glomerular thrombotic microangiopathy (TMA) while increased production leads to collapsing glomerulopathy. Complement activation and genetic variants in regulatory complement factor H (CFH) are also features of both ARMD and glomerular TMA associated with hemolytic uremic syndrome (HUS). Therefore, we hypothesized that VEGF and CFH may interact. Using cell culture, mouse models and patient samples, we show that VEGF inhibition, the main therapy used to treat wet ARMD, reduces local CFH and other regulatory complement proteins leading to complement activation in the eye and kidney. This is mediated by reduced VEGFR2-PKC-CREB signalling. Human cells carrying ARMD- or HUS-associated CFH genetic variants show more alternative complement pathway activation than controls. This is increased by VEGF antagonism, suggesting patients carrying these genetic variants may be more susceptible to complement overactivation due to VEGF inhibition. Furthermore, complement inhibition partially reduced anti-VEGF induced endothelial cell activation. Though further study is warranted, these findings suggest an individualized approach to the use of VEGF antagonists with greater patient monitoring of both retinal and renal function should be considered, as prolonged use of VEGF inhibitors in some patients may result in complement-mediated tissue damage.

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In-depth diagnostic immune-profiling of two X-linked Severe Combined Immune Deficiency (SCID) siblings identified through the Illinois newborn SCID screening program

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Mandatory statewide newborn screening for T cell-deficient Severe Combined Immunodeficiency (SCID) was implemented in Illinois in January 2014. This entails molecular analysis for

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T cell Receptor Rearrangement Excision Circles (TREC) from heel-stick derived dried blood spots collected at birth at the state public health laboratory followed by confirmatory flow-cytometry testing at our institution's CLIA and CAP-certified diagnostic immunology laboratory (and a Jeffrey Modell Foundation designated Diagnostic and Research Laboratory for Primary Immunodeficiency Diseases) for neonates that register < 250 TRECs/microliter of blood. In July 2014, we analyzed a peripheral blood sample from a male neonate with a TREC count of 0. Flow-cytometry revealed markedly reduced numbers of T cells and Natural Killer cells but normal B cell counts, raising the possibility of T-B+NK- SCID, arising due to a mutation in either the gene encoding for the common gamma chain [CD132; signaling sub-unit shared by the receptors for IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21 (X-linked SCID)] or the Janus Kinase 3 gene [JAK3 (Autosomal Recessive SCID)]. Phenotypic analysis of the few T cells available revealed a preponderance of a memory phenotype as well as normal surface expression of CD132 and cytosolic expression of JAK3. However, functional immune profiling demonstrated depressed phytohemagglutinin-induced proliferation of the T cells, as well as marked hypo-phosphorylation of B cell-associated STAT6 following IL-4 treatment, suggestive of a severe attenuation of the signaling potential in the CD132-JAK3 axis. Subsequent sequencing of the patient's genomic DNA revealed a non-sense mutation in the CD132 gene [(c.375C>A); (p.Y125X)], which would have resulted in the production of a common gamma chain completely lacking the transmembrane and intracellular domains, confirming the prediction based on the immune profiling results. In September 2015, our index patient's infant brother was referred to our institution following his abnormal newborn screen, also with a TREC count of 0. Phenotypic and functional assessment of his lymphocyte subsets demonstrated an immune profile that was almost exactly the same as that of his older brother. After the diagnosis of X-linked SCID was made in each case, the siblings underwent unrelated donor stem cell transplant following reduced-intensity conditioning with fludarabine, busulfan and anti-thymocyte globulin. Both siblings attained full donor engraftment and immune reconstitution after the transplant and are now clinically well. In conclusion, functional immune profiling of patients' lymphocytes, including phosphorylation studies of receptor signaling molecules, is a rapid and effective way to diagnose immunodeficiency and should be considered a routine part of the work-up for newborns with possible SCID.

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Blocking viral access to heparan sulfate reduces 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. 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, 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 alone is sufficient for binding, and this interaction requires the presence of the glycosaminoglycan heparan sulfate (HS), a repeating sulfated disaccharide sugar found on HS proteoglycans. 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 several enveloped viruses, without anti-coagulant activity *in vivo*. Peptide dendrimer SB105-A10 specifically interacts with HS chains on the cell surface and occludes any further ligand binding, such as viral particles. We found the pretreatment of virus with highly sulfated K5 polysaccharides, specifically those sulfated at position C6 of the glucosamine sugar, inhibited HMPV infection in multiple human lung cell lines, suggesting negative charges from the sulfation of HS are critical for interaction with the HMPV F protein. Furthermore, these results suggest the C6 sulfation position is a key feature of HS necessary for HMPV interaction and possibly other viruses that use this mammalian sugar to bind their target cells. Peptide dendrimer SB105-A10 inhibited HMPV infection as well, suggesting occlusion of HS at the target cell surface is sufficient to prevent infection. We demonstrated this decrease in infection is a result of inhibition of particle binding, and the interaction is dependent on the F protein as mutant viruses lacking the other two envelope proteins also were blocked from binding. These results were also seen in a 3D model using Human Airway Epithelium (HAE) tissues, suggesting these interactions take place during HMPV infection in a physiologically relevant model that recapitulates the complexity of the human airway. Thus, these compounds serve as a platform for potential antiviral development.

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Insights into Parkin ligase mechanism from structures of a minimal Miro substrate

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Hereditary Parkinson's disease is predominantly caused by mutations in the protein kinase PINK1 or the E3 ubiquitin ligase Parkin, which function together to eliminate damaged mitochondria. PINK1 phosphorylates both Parkin and ubiquitin to stimulate ubiquitination of dozens of proteins on the outer surface of the mitochondrial membrane. However, the mechanisms by which proteins targeted by Parkin are selected for modification remain largely unexplored. Here,

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we characterize the Parkin-mediated ubiquitination of human Miro1 and Miro2 (hMiro1/2). Guided by crystal structures of several hMiro1/2 domains, we show that that the C-terminal GTPase (cGTPase) is necessary and sufficient for efficient hMiro ubiquitination. Sidechain location, chemical microenvironment, and substrate oligomeric state act as determinants for lysine modification. Finally, we find that phosphorylation of Parkin and ubiquitin activate preferential targeting of a specific lysine, K572, in the hMiro1 cGTPase, suggesting a dual role for Parkin phosphorylation: catalytic stimulation and substrate lysine prioritization. Our findings establish a structural and mechanistic framework for understanding the specificity of primary substrate ubiquitination by Parkin.

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Epigenetic Antagonism of Ezh2 and Jmjd3 in Microglia Polarization

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Microglia are the myeloid-derived resident innate immune cells of the brain that serve to maintain homeostasis of the central nervous system (CNS). Brain injury, such as ischemic stroke, activates microglia which may polarize to either a pro-inflammatory (M1) or anti-inflammatory (M2) phenotype. However, while microglia have been shown to be predominantly anti-inflammatory M2 early after stroke *in vivo*, the direct effects of ischemia on microglia polarization and the underlying epigenetic regulation are unknown. Recent work has shown that Jumonji Domain Containing 3 (Jmjd3, KDM6B) is essential for epigenetically regulating anti-inflammatory M2 polarization. Jmjd3 demethylates trimethylation marks on histone H3 lysine 27 (H3K27me3) to monomethylation marks (H3K27me1). This relaxes chromatin and allows for gene transcription to occur. The actions of Jmjd3 may be antagonized by Enhancer of Zeste Homologue 2 (Ezh2), a histone methyltransferase. Ezh2 functions to establish H3K27me3 marks, thereby causing condensation of chromatin and subsequent repression of gene transcription. We hypothesized that inhibition of Ezh2 may tilt the balance in favor of higher overall Jmjd3 activity, thereby enhancing polarization toward an anti-inflammatory M2 phenotype. Furthermore, we hypothesized that oxygen glucose deprivation (OGD), an *in vitro* model of ischemia, directly induces anti-inflammatory M2 polarization of primary microglia. We used primary microglia cultures from individual male and female neonatal mice and pretreated them with GSK343 (a functional inhibitor of Ezh2) or vehicular control. We then stimulated them with LPS+IFNα or IL-4, stimuli for M1 and M2 phenotypes, respectively. Treatment with GSK343 significantly up-regulated M2-associated genes CD206, IRF4, and ARG1 at baseline, enhanced their expression following M2 stimulation, and rescued their otherwise down-regulation following M1 stimulation. In chromatin immunoprecipitation (ChIP) assays, we found that Ezh2 was recruited to these gene promoters and established H3K27me3 marks following M1 stimulation. Additionally, treatment with GSK343 suppressed up-regulation of M1-associated genes IL1B, IL6, TNFA, and NOS2 following M1 stimulation. These data suggest that Ezh2 simultaneously promotes pro-inflammatory M1 polarization and antagonizes Jmjd3 activity to suppress M2 polarization. Interestingly, in our model of OGD, qPCR analysis revealed up-regulation of both KDM6B and EZH2 (6-fold, $p<0.001$ and 3-fold, $p<0.01$, respectively). Consistent with this observed 2:1 KDM6B:EZH2 expression ratio, assessment by qPCR of a panel of M1- and M2-associated genes revealed a predominantly anti-inflammatory M2 phenotype following OGD. This also corresponded with multiplex cytokine analysis of cell culture supernatant of OGD-treated samples. These findings suggest epigenetic antagonism of Ezh2 and Jmjd3 in microglia polarization, and that Jmjd3 plays a significant role in promoting anti-inflammatory M2 microglia polarization following OGD.

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Real-Time Assessment of Fatty Acid Trafficking In The Human Placenta

WITHDRAWN

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The fetus requires large quantities of long-chain fatty acids to serve as building blocks for the developing nervous and cardiovascular systems. If the maternal supply of these essential molecules is limited, the fetus will suffer cardiovascular deficits and cognitive dysfunction. Maternal dietary deficiencies or abnormal transport systems in the placenta can deprive the fetus of needed bio-lipids. Despite being crucial for normal human development, little is known about the mechanisms underlying the transport of lipids in placenta. Maternal lipids must cross three cellular layers to reach the fetus: the syncytiotrophoblast, the cytotrophoblast and the fetal capillary endothelium. We used live-confocal fluorescence microscopy to track the movement of the fluorescently conjugated fatty acid analogues of various chain-lengths, BODIPY-C5, BODIPY-C12, and BODIPY-C16, across these cell layers of living explants of human term placenta. We found the trafficking of fatty acids through placental tissue was chain-length dependent. When fatty acids were added to the maternal compartment, the mean uptake rate into the fetal capillary space for 5-Carbon, 12-Carbon, and 16-Carbon FA was 79±7, 42±0.2, 13±0.1 Fluorescence Units (FU) min-1, respectively. In the first layer (syncytiotrophoblast) the 5-Carbon, 12-Carbon, and 16-Carbon FA uptake rate was 53±2, 37±1, 50±1 FUmin-1, respectively. Furthermore, fatty acid uptake appears to differ by placental cell type and fatty acid species. For example, rapid esterification of the long-chain fatty acid analogue and incorporation into lipid droplets was exclusive to the inner layer cytotrophoblast cells rather than the expected outer syncytiotrophoblast layer. Syncytiotrophoblast accumulates very-long chain fatty acid BODIPY-C16 and rapidly effluxes the shorter BODIPY-C5 fatty acid. Isolated cytotrophoblast and differentiated syncytiotrophoblast recapitulate these phenomena in culture; syncytialized cells abandon their long-chain fatty acid metabolic capacity. Differentiation of cytotrophoblast leads to suppression of previously active genes that regulate fatty-acid

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uptake (SLC27A2/FATP2, FABP4, ACSL5) and lipid metabolism (GPAT3, LPCAT3). The cytotrophoblast progenitor cell layer was not previously recognized as being important in placental fatty acid uptake and metabolism. We speculate that cytotrophoblast performs a vital role in placental fat metabolism; it may also regulate fat transport to the fetus.

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AAV-expressed eCD4-Ig provides durable protection of rhesus macaques from multiple SHIV challenges

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Long-term *in vivo* expression of a broad and potent entry inhibitor could circumvent the need for a conventional vaccine for HIV-1. Adeno-associated virus (AAV) vectors can stably express HIV-1 broadly neutralizing antibodies (bNAbs). However, a large fraction of HIV-1 isolates remain partially or wholly resistant to even the best bNAbs (80% inhibitory concentration (IC80) > 5 mg/ml). Higher concentrations will probably be necessary for general protection, but these concentrations may be difficult to establish in humans. We show that eCD4-Ig, a fusion of CD4-Ig with a small CCR5-mimetic sulfopeptide, binds avidly and cooperatively to the HIV-1 envelope glycoprotein (Env) and is more potent than the best bNAbs (geometric mean half-maximum inhibitory concentration (IC50) < 0.05 mg/ml). Because eCD4-Ig binds only conserved regions of Env, it is also much broader than any bNAb. For example, eCD4-Ig efficiently neutralized 100% of a diverse panel of neutralization- resistant HIV-1, HIV-2 and simian immunodeficiency virus isolates, including a comprehensive set of isolates resistant to the CD4-binding site bNAbs VRC01, NIH45-46 and 3BNC117. Rhesus macaques inoculated with an AAV vector stably expressed 17–77 mg/ml of fully functional rhesus eCD4-Ig for more than 40 weeks, and these macaques were protected from several infectious challenges with SHIV-AD8. Rhesus eCD4-Ig was also less immunogenic than rhesus forms of four well-characterized bNAbs. Our data suggest that AAV-delivered eCD4-Ig can function like an effective HIV-1 vaccine.

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Kir6.1 limits peri-infarct depolarization frequency during focal cerebral ischemia

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Objective: Focal cerebral ischemia results in recurring spreading depression-like events initiated within the ischemic core, leading to peri-infarct depolarizations (PIDs) in the surrounding tissue. PIDs are thought to exacerbate ischemia, expanding the core and further contributing to neuronal death. Pathologic increases in extracellular potassium concentration due to anoxic K+ release play a critical role in PID initiation and propagation, but the molecular mechanisms driving PID phenomena are poorly understood. ATP-sensitive potassium channels (KATP), composed of pore-forming (Kir6.1 or Kir6.2) and regulatory sulfonylurea receptor (SUR1 or SUR2) subunits, open under low ATP conditions (e.g. ischemia) and facilitate transmembrane K+ fluxes. Both Kir6.1 and Kir6.2 are expressed in neural tissue, including neurons, astrocytes, and endothelial cells, and thus these channels may be critical for maintenance of extracellular K+ homeostasis during ischemia. We sought to determine the role of these channels in PID occurrence during ischemia. **Methods:** Male mice (wild-type, Kir6.1-/-, or Kir6.2-/-, on a C57Bl6/J background) were anesthetized with isoflurane and middle cerebral artery occlusion was achieved by intra-arterial filament. The scalp was retracted and wide-field OIS imaging was performed through the intact skull for 4 hours. LEDs operating at four wavelengths illuminated the skull, and diffuse reflected light was detected by EMCCD camera. PIDs were scored by manual viewing of data. PID frequency was compared between groups using ANOVA with repeated measures and Newman-Keuls multiple comparisons test. **Results:** Ischemia in wild-type mice resulted in PID initiation 39±9 minutes into ischemia. PIDs continued to occur throughout the 4-hour imaging window at a consistent frequency of 2.8±1.0 PIDs/hr. In Kir6.1-/- mice, PID initiation was earlier (21±6 min vs. 39±9 min, $p<0.05$), and occurred with increased frequency compared to control mice (5.0±1.8 PIDs/hr vs. 2.8±1.0 PIDs/hr, $p<0.05$). In Kir6.2-/- mice, PID initiation time and frequency were unchanged compared to controls (34±14 min vs. 39±9 min, N.S.; 2.6±1.7 PIDs/hr vs. 2.8±1.0 PIDs/hr, N.S.). **Conclusion:** These results demonstrate a critical role for Kir6.1 in limiting PIDs during ischemia. Given relevant activation of this channel during ischemia, it may play a role in removing excess extracellular K+. Previous studies have shown that Kir6.1 gene deletion results in increased infarct volumes [5], consistent with the idea that PIDs contribute to infarction. Kir6.1 and Kir6.2 exhibit different cell-type distributions [4], suggesting KATP channel activation in a specific cell type may be necessary for limiting PID. We are currently working to identify that cell type in order to better understand how KATP channels limit ischemic depolarization and neuronal death.

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Vitamin D reduces progerin expression and rescues genomic instability and premature senescence in Hutchinson-Gilford Progeria Syndrome

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Hutchinson-Gilford Progeria Syndrome (HGPS) is a devastating premature aging disease, leading to patient death by the end of their teenage years. HGPS is caused by mutations in the LMNA gene, preventing normal processing of lamin A and producing a toxic product called progerin. Progerin elicits profound nuclear morphological abnormalities, altered cell signaling, genomic instability, and other cellular disturbances, all of which contribute to disease. New treatment strategies are desperately needed for treating this universally fatal disease since current therapies offer only modest benefits with significant side effects. Our analysis of the LMNA gene locus identified a Vitamin D Receptor (VDR) binding element downstream of the transcriptional start site. Interestingly, ligand activation of VDR in five hematopoietic cell lines results in decreased VDR occupancy of this regulatory element and decreased LMNA gene expression, suggesting that vitamin D could represent a strategy for reducing toxic progerin expression in HGPS. Here, we demonstrate that VDR maintains basal expression of LMNA in normal fibroblasts and of progerin in HGPS patient-derived fibroblasts. Importantly, ligand activation of VDR results in a marked downregulation of progerin in HGPS cells. As a consequence, prolonged treatment of HGPS cells with 1,25D (the most bioactive vitamin D metabolite) dramatically reduces the characteristic accumulation of progerin in these cells in culture and ameliorates a variety of cellular phenotypes, including nuclear morphological abnormalities, accumulation of DNA damage, and premature entry into senescence. Vitamin D/1,25D emerges from these studies as an exciting therapeutic possibility for HGPS and potentially other laminopathies caused by mutations in the LMNA gene.

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Prioritization of Genetic Variants using Annotation Aware Trans-ethnic Fine-Mapping in Rheumatoid Arthritis

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Introduction: The inability to separate candidate causal variants from neutral genetic variants passively associated with a disease is a major contemporary obstacle to the study of the genetics of complex disease. Genome wide association studies (GWAS) tend to identify dozens of associated genetic variants per locus all of which are in linkage with a smaller number of causal variants. However, due to different haplotype structure and linkage between variants, trans-ethnic fine-mapping approaches can be used to prioritize potential causative variants and increase so-called "fine mapping power." We propose meta-analysis and fine-mapping of Rheumatoid Arthritis (RA) risk loci using genetic data from four global populations: Asians, Caucasians, African Americans, and South Africans to prioritize risk variants in each genetic locus. **Methods:** We have association summary statistics (Z-scores) from GWAS of 4 different populations of patients with RA, as well as LD information either calculatee using 1000 genomes data or calculated empirically using our data. In addition, we have taken functional annotation information from sources such as ENCODE, ROADMAP, and Gusev et al. 2014. We will integrate this information using PAINTOR2, a trans-ethnic fine-mapping algorithm capable of modeling more than one risk variant per locus. We then applied this algorithm to over 80 RA risk loci in order to prioritize genetic variants by their posterior probability of being causal variants. **Results:** Our approach identified several dozen genetic variants with posterior probability > 0.99 of being causal variants. For instance, we identified rs7565213 and rs11571302 in the CD28/CTLA4 locus with posterior probability of causal > 0.999. Other loci of interest include AFF3, TNFAIP3, TAGAP, NFKBIE, and CCR6. **Conclusions:** Our rich troves of genetic data on RA combined with this approach can provide RA researchers have identified high priority candidate causal genetic variants in several dozen RA risk loci. We present these as a service to the field, in order to increase cost-efficacy of validation studies designed to establish the functional role of associated risk variants. Overall, this study uses a cutting-edge approach to address a major obstacle to progress in understanding the genetic architecture of complex disease: finding an effective method of sifting through associated variants to find causal variants at risk loci. These results underscore the importance of publishing GWAS association summary statistics for use in meta-analysis.

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Investigating combination therapies of robot-driven epidural stimulation, robot rehabilitation, and viral delivery of Brain-derived neurotrophic factor (BDNF) in treating adult spinal cord injury (SCI)

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A complete transection at T10 in an adult rat (ATX) is a useful model to investigate locomotor rehabilitation in SCI, where we employ a unique trunk-based robotic intervention to improve locomotor function with treadmill training in various treatment paradigms, such as epidural stimulation. We hypothesize our robot can exploit rudimentary hindlimb stepping patterns for rehabilitation. Previously, we showed that AAV5 viral delivery of BDNF in the ATX model significantly improves locomotor recovery when combined with our robot. Thus, we propose a

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combined treatment regimen of epidural stimulation, robot rehabilitation, and AAV5-BDNF to investigate combination therapies. We prepared three groups of rats (n=11) with stimulating electrodes placed on the spinal cord at L2 and S2, and microinjections caudal to injury into the spinal cord: two groups receiving AAV5-BDNF, and another receiving a sham virus. The control and one BDNF group were treadmill trained with robot and epidural stimulation for six weeks. The other group was treadmill trained with epidural stimulation, but without active robot assistance, during the same time. To examine the effects of combination therapy between groups, we compared (1) stimulation intensity needed to induce locomotor activity, and (2) the threshold to induce locomotor activity as a function of time during recovery. We discovered that the BDNF groups showed (1) significantly less stimulation intensity needed to induce locomotor function, which (2) lasted throughout the rehabilitation, as compared to the control group. This work is sponsored by the Craig H. Neilsen Foundation, the NS 54894, and the Drexel College of Medicine Dean's Fellowship for Excellence in Collaborative or Themed Research.

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Loss of somatostatin receptor 2 expression reduces small cell lung cancer growth and alters cellular metabolism

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Small cell lung cancer (SCLC) is a high grade poorly differentiated neuroendocrine carcinoma of the lung responsible for ~15% of diagnosed lung cancers and up to 25% of lung cancer deaths. Treatment paradigms in small cell lung cancer have not changed significantly in the last 20 years. Advances in targeted therapies for SCLC are sorely needed. Somatostatin receptors (SSTR) are neuroendocrine associated G protein-coupled receptors associated with multiple tumor types and which have effects on cell cycling, angiogenesis, apoptosis, and growth factors. They canonically signal by inhibition of adenylate cyclase, calcium influx, and act through downstream MAPK and Akt as well as other downstream kinases. We evaluated SSTR2 expression by IHC staining and western blotting of multiple cell lines and tumor tissues and found high expression in multiple neuroendocrine lung carcinomas including classical and variant SCLC lines. Given the high level of SSTR2 expression found in most SCLC lines, we hypothesized that this signaling pathway stimulates growth and survival of these tumor cells. We followed these preliminary studies with an assessment of 98 SCLC patients whose tumor IHC was subdivided into high or low SSTR2 expressing tumors by continuous IHC scoring. Low SSTR2 tumors had a better prognosis with a median survival in limited stage disease of 36 months compared to 12 months in the SSTR2 high expressing SCLCs suggesting that SSTR2 expression has clinical relevance in SCLC progression. The hazard ratio was 0.45 with a $p<0.05$. This led to further experiments to interrogate the mechanism and function of SSTR2 in SCLC. We established multiple stable SSTR2 shRNA knockdown lines including bronchial carcinoid, and multiple adherent SCLC lines. SSTR2 knockdown led to up to 3 fold changes in cell viability *in vitro* with reduced proliferation in multiple cell culture lines and constructs as well as apoptosis in H1048 cells. Metabolic testing with Seahorse mito stress kits concurred with significant differences in mitochondrial respiration and metabolic activity in the SSTR2 knockdown cell lines compared to the control scrambled construct cell line. We are actively pursuing metabolic studies in SCLC and will expand this work to evaluate ADP/ATP and AMP/ATP ratios to determine if aberrant energy homeostasis may contribute to the observed cell death in this metabolically active cell population. Collectively, this data brings new interest to SSTR2 signaling as a pathway target for therapy in a subset of SCLC and suggests potential metabolic targets in SCLC.

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Inflammation in a Mouse Model of Acute Kidney Injury to Chronic Kidney Disease Transition

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Introduction: Ischemic acute kidney injury (AKI) increases risk of morbidity and mortality and is highly prevalent in hospitalized patients, those with hemodynamic instability, and the elderly. AKI has been associated with increased risk of chronic kidney disease (CKD). Our laboratory has previously demonstrated a pivotal role for myeloid cells and heme oxygenase-1 in modulating damage from ischemic AKI. Objectives: We sought to determine the role of renal mononuclear cells in the development of fibrosis, which occurs late after AKI. We used a murine model in which injury is induced by 30 minutes left kidney ischemia, while leaving the right kidney intact. This procedure is known to induce fibrosis by 3 weeks post-AKI in the ischemic kidney. The changes in inflammatory resident mononuclear phagocytic and infiltrating cells in terms of function and phenotype are unknown in this model. **Methods:** C57BL/6 mice were subjected to 30 minutes of ischemia followed by reperfusion. Tissues were harvested at 1 week and 3 weeks post-ischemia and were compared with sham controls. Flow cytometry, histology, and protein analysis allowed investigation of the immune phenotype, and fibrotic and morphologic change. **Results:** 30 min unilateral renal ischemia in C57BL/6 x FVB mixed background mice caused minor inflammation, collagen deposition, and tubular dilatation. Absolute number of neutrophils remained significantly increased in the injured kidney 1 week post-ischemia, while other cell types trended toward an increase. **Conclusion:** Future studies will focus on the oxidative stress and immune response during the AKI to CKD transition in this model. This will help elucidate the role of inflammation in modulating disease progression and provide avenues of investigation for therapeutic intervention.

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UDP-xylose transport in Cryptococcus neoformans virulence

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Cryptococcus neoformans is an opportunistic fungal pathogen that infects more than one million people annually, killing over 600,000 of these individuals worldwide. Glycoconjugates are crucial determinants of cryptococcal homeostasis and pathogenesis, making these carbohydrate structures attractive therapeutic targets, but our knowledge about how they are synthesized is limited. Glycan modifications of cryptococcal proteins and lipids incorporate significant amounts of xylose. This monosaccharide also constitutes over one-fourth of the mass of the polysaccharide capsule, the definitive cryptococcal virulence factor that surrounds the cell. Incorporation of sugar moieties into these cellular carbohydrate structures requires activated donors, usually nucleotide sugars like the UDP-xylose that serves as the xylose donor for synthetic reactions. While nucleotide sugars are generally synthesized in the cytosol, only a small fraction of these precursors is consumed there. The majority is translocated into the secretory pathway where most glycan biosynthesis occurs to modify critical proteins and lipids, and form the protective capsule. Nucleotide sugar transporters (NSTs) are thus required to transport the raw materials to the site of synthesis to preserve virulence and cellular viability. However, despite their key role in glycan synthesis, the identity and regulation of the complete set of cryptococcal NSTs remains elusive. We identified two putative UDP-xylose transporters in C. neoformans, NSTN and NSTM. Although the two proteins are differentially expressed in response to environmental conditions, several lines of evidence suggest that they are functionally redundant. First, biochemical analysis of capsule glycoconjugates synthesized in the luminal compartments showed a decreased percentage of xylose in nstM, and a complete absence of xylose in the double mutant. Also, while deletion of NSTM or NSTN alone did not appreciably alter capsule size, stress sensitivity, or virulence in mice, deletion of both genes, resulted in significant growth defects, altered capsule morphology, and decreased survival in macrophages. Furthermore, nstM nstN was unable to cause disease in mice despite long-term persistence in the lungs. Defining the activity of NSTM and NSTN will advance our understanding of glycan biosynthesis, setting the stage for further studies of fundamental glycobiology and cryptococcal pathogenesis.

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Microscopic image guidance: real-time thermal therapy monitoring in Barrett's esophagus

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The term "image-guided therapy" has traditionally been confined to predominantly macroscopic imaging modalities (such as CT, MR, and US, which offer a resolution on the order of millimeters) for guiding therapeutic interventions. Here, we introduce the concept of microscopic image guidance for real-time thermal therapy monitoring in epithelial lesions using optical frequency domain imaging (OFDI), which provides tissue microstructural details at high resolution (~10 μm) in 3D. In our clinical studies, catheter-based OFDI has shown promise in a number of cardiovascular and GI applications including Barrett's esophagus (BE) screening. In particular, BE with high-grade dysplasia (HGD) carries a significant risk of progression to esophageal adenocarcinoma (5-yr survival: ~15%). While radiofrequency ablation (RFA) is now an established treatment modality for BE with HGD, its use remains limited for earlier stages. An important obstacle is that these RFA procedures can be highly variable as they incorporate minimal guidance for lesion identification using only macroscopic surface features assessed by endoscopy and rely on pre-determined energy settings for dose delivery (assuming uniform lesion depth). To solve this problem, we propose an OFDI-guided and OFDI-monitored thermal therapy platform. In this work, we demonstrate the use of dynamic OFDI phase measurements, called complex differential variance (CDV), for real-time therapy monitoring, which enables the direct, non-invasive assessment of the coagulation zone at high resolution. By contrast, conventional RFA monitoring based on temperature is often invasive, limited by point sampling, and are indirect measures of tissue injury, while emerging techniques such as MR and US thermometry are limited by their spatial resolution. For epithelial applications, the ability to visualize the thermal injury zone at high resolution is critical to the precise delivery of thermal energy to the target lesion. We showed that our technique can accurately delineate the coagulation zone in multiple tissue types including the esophagus, skin, and retina in our ex vivo validation study and we are currently translating this technique for testing in a catheter-based setting to enable *in vivo* experiments in the future. The ability to delineate thermal lesions in multiple tissue types opens up the possibility of performing microscopic image-guided procedures in a vast array of epithelial applications, such as laser therapy in hypertrophic scars and diabetic retinopathy, in addition to RFA in BE.

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Optogenetic Stimulation of EphB2 Signaling in Dendrites Promotes Nucleation of Dendritic Actin Networks

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Dendritic spines are mushroom-shaped, actin-based protrusions that comprise most excitatory post-synaptic compartments in the central nervous system. Many neurodevelopmental and neurodegenerative diseases are associated with abnormal spine density and morphology. Spines are thought to form from thin, dynamic protrusions known as dendritic filopodia, which may transform into spines upon contact with axons. Eph receptors, specifically EphB2, are thought to mediate the transition from dendritic filopodia to spines. The goal of this study is to understand how local EphB2 signaling, which occurs at axo-dendritic contacts, affects the actin cytoskeleton in filopodia. While the EphB2 signaling pathways in dendrites have been well-studied, the spatial and temporal characteristics of signaling are poorly understood. Ligand-mediated stimulation additionally does not provide a good model for local axo-dendritic contact. We developed an optogenetic tool for EphB2 signaling, optoEphB2, to probe for changes in dendritic protrusions with targeted EphB2 stimulation. OptoEphB2 uses the blue light-induced clustering of the plant photoreceptor cryptochrome 2 to achieve EphB2 tyrosine kinase activation. Initial experiments in 3T3 cells demonstrated that blue light-induced optoEphB2 clustering resulted in increased tyrosine phosphorylation, recruitment of SH2 domains that typically bind endogenous EphB2, and cell collapse, thereby validating normal EphB2 signaling. Focal illumination of cell processes showed spatially-restricted clustering and collapse, confirming spatial control. We then transfected cultured hippocampal neurons with optoEphB2 and focally illuminated dendritic filopodia to mimic contact with axons. We found that optoEphB2 induced branch formation in filopodia, which depended on the actin nucleators Arp2/3 and N-WASP. Axons may also contact the dendritic shaft, and optoEphB2 stimulation in the dendritic shaft resulted in the spatially-restricted formation of new filopodia, dependent on Arp2/3, N-WASP, PI3K, and Arg. Presumably, Arg may locally stabilize the binding of Arp2/3 and N-WASP to actin, and PI3K activity may also recruit N-WASP. This study therefore provided new insights into EphB2 regulation of dendritic protrusions. We conclude that EphB signaling promotes branched actin networks in dendrites. This may contribute to the formation of new filopodia and, therefore, new synaptic spines, or may have implications for the formation of the highly-branched cytoskeleton in the spine head. Additionally, optoEphB2 is a versatile module and may be used in other systems to study the spatial and temporal aspects of EphB2 signaling.

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Cell density organizes collective migration through changes in actomyosin contractility

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Collective migration of cells underlies embryonic development, tissue regeneration, and tumor invasion. Despite this widespread importance, there is still an incomplete understanding of the physical and biological mechanisms that allow multiple cells to organize their motion and furthermore, how these properties shape the specific types of collective migration that emerge. Using iterative hypothesis testing between simulation and experiment, we track the motion of thousands of cells to determine how the fundamental properties of cell density, adhesion, and actomyosin contractility combine to produce collective migration. We find that adhesion competent cells undergo an initial paradoxical decrease in organization with increasing density followed by an increase in organization at the highest densities. This trend reflects two processes. First, the high degree of organization at low density is produced by regulated actomyosin contractility. At low density, organization exhibits a biphasic response to contractility: enhancing or diminishing actomyosin contractility or uncoupling cells through by reducing cell-cell adhesion reduces organization. Second, the organization that appears at high density is consistent with a cell-cell packing mechanisms that arises through adhesion and contractility independent mechanisms. Furthermore these two mechanisms of organization produce collectives with distinct patterns. Contractility mediated collective migration produces broad collective groups with cells moving in line with neighbors to either side. Packing mediated collective migration results in a pattern known as cell streaming with cells following those in front and sliding past neighbors on either side. We test these predictions in-vivo using the drosophila ovarian follicular epithelium, a population of cells that undergoes a highly organized motion during egg development, providing insights into the initiation and maintenance of motion during this process.

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Elucidating the genetic determinants for stem cell exit out of pluripotency with a CRISPR-cas9 genome-wide knockout screen

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Pluripotent stem cells in the early mammalian embryo progress through developmental states in preparation for lineage specification during gastrulation. Two distinct states of pluripotency can be replicated *in vitro* by using mouse embryonic stem cells (ES) for a naïve state and epiblast like cells (EpiLC) for a primed state. Naïve ES cells can rapidly transition to the EpiLC primed state with simple changes to cell culture

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media. This transition is irreversible, so EpiLC die if they are returned to naïve cell culture conditions. Previously, several genes have been shown to be necessary for the transition from naïve to primed states, and inactivation of these genes prevents cell death when cells are switched between EpiLC and ES culture conditions. To screen for novel genes required for exit out of naïve pluripotency, I performed a CRISPR-cas9 genome-wide pooled knockout screen and targeted all protein coding genes in the mouse genome with ~90k unique sgRNAs. The screen yielded 30 high confidence candidates (FDR<5%), including genes known to be required for naïve to primed transition, such as: Tcf7L1, Zfp281, Tsc1, Tsc2, and Flcn. Novel genes were also identified; interestingly, many of these genes affect endocytic trafficking to the lysosome. There was a particularly strong enrichment for genes that are part for the HOPS complex important for late endolysosome fusion and mTOR pathway genes involved in amino acid sensing on lysosomes. Here, we identify known and novel genes that provide new hypotheses aimed at gaining greater understanding of the naïve to primed pluripotency transition.

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The Signaling Protein TRAF3 Regulates the Metabolic Profile of B cells

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TRAF3 is an adaptor protein that negatively regulates signaling through CD40 and BAFF receptors in B lymphocytes. B cells from B cell conditional TRAF3-/- mice display a remarkable pro-survival phenotype compared to wild type B cells, but the mechanism for this abnormal survival is poorly understood. We find that loss of TRAF3 leads to increased uptake of glucose in B cells *in vitro* and *in vivo*. This is accompanied by increased oxidative phosphorylation and glycolysis. In the absence of TRAF3, expression of Glut1 and Hexokinase II, two molecules important for glucose metabolism, is increased. Treatment of B cells with Glut1 inhibitor or competitive glycolysis inhibitor 2-DG attenuates survival and B cell viability is substantially reduced in a glucose free environment. Deletion of the MAP kinase NIK reverses the increase in glucose uptake and Glut1 expression in TRAF3-deficient B cells, highlighting the significance of TRAF3-mediated NIK regulation in B cell metabolism.

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Inhibiting DNA-PK Radiosensitizes Human Osteosarcoma and Chondrosarcoma Cells

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Primary bone tumors such as osteosarcoma and chondrosarcoma are mainly treated with surgical intervention. When these tumors are located in difficult regions such as the pelvis, surgical intervention could lead to major side effects such as loss of a limb and/or function, loss of bowel, bladder and sexual function. Radiation therapy is effective only in one of these tumors - Ewing's Sarcoma. Enhanced DNA repair is one of the major reasons why these tumors are radio-resistant. Hence, targeting DNA repair could be an effective strategy to radiosensitize them. Non-homologous end joining (NHEJ) is considered to be the major DNA repair pathway for radiation-induced DNA damage. DNA-dependent protein kinase (DNA-PK) is an essential component of NHEJ. Hence, the goal of this study was to investigate the efficacy of the DNA-PK inhibitor, KU60648, in radiosensitizing various primary bone tumor cell lines. Western Blotting was used to confirm expression of DNA-PK and inhibition of DNA-PK autophosphorylation by KU60648. The radiosensitizing effect of KU60648 was investigated in two human osteosarcoma cell lines, 143B and U2OS, and in a chondrosarcoma cell line SW1353. DNA damage was assessed using immunofluorescence based gamma-H2AX foci assay. Western Blotting showed that DNA-PK is highly expressed in the various primary bone tumor cell lines investigated. Co-treatment with KU60648 and radiation effectively decreased the clonogenic survival of these tumor cells compared to radiation alone. Combining KU60648 and radiation also led to an increased accumulation of the tumor cells in G2/M arrest and enhanced the proportion of cell nuclei with gamma-H2AX foci compared to using radiation alone. Moreover, DNA-PK autophosphorylation (at Serine 2056) was inhibited by KU60648 in a concentration dependent manner. We conclude that inhibiting DNA-PK is an effective strategy that can be combined with other approaches for radiosensitizing primary bone tumors such as osteosarcoma and chondrosarcoma.

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Redox profiling of carvedilol and propranolol in H9C2 cardiomyocytes

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Clinical trials have shown that carvedilol is highly effective against heart failure (HF). Carvedilol, unlike propranolol, has direct antioxidant effects and is capable of mitigating oxidative stress in HF patients. Moreover, it has been suggested that carvedilol has an indirect antioxidant mechanism that could involve the initial production of non-lethal levels of oxidative stress leading to the regulation of an uncharacterized antioxidant response that later counters oxidative stress. We hypothesized that carvedilol's indirect antioxidant mechanism may involve the nuclear factor erythroid 2-related factor 2 (Nrf2)/Kelch ECH associating protein 1 (Keap1) pathway, which is a major antioxidant pathway involved in cardiovascular, pulmonary, and neoplastic diseases. Using H9C2 cardiomyocytes, we confirmed the activation of the Nrf2/Keap1 pathway by detecting levels of downstream protein targets hemeoxygenase-1 (HO-1) and NAD(P)H: quinone oxidoreductase-1 (NQO-1). We transfected H9C2 cells with reductive-oxidative green fluorescent protein (roGFP) fused with human glutaredoxin 1 that targeted

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mitochondria or cytosol. Redox state changes were quantified by normalized roGFP intensity ratios measured using live-cell imaging. In the short term, carvedilol oxidized both cellular compartments while propranolol did not. In the long term, carvedilol upregulated the production of HO-1 and NQO-1 while propranolol downregulated these antioxidant proteins. These results demonstrate that carvedilol's indirect antioxidant effect involves the Nrf2/Keap 1 pathway. This has strong implications as carvedilol is a commonly used, highly effective beta-blocker and elucidating its antioxidant mechanisms can potentially expand the use of carvedilol for the treatment of other diseases, inform the development of new therapeutics, and optimize HF treatment.

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GM-CSF induced bone-marrow derived dendritic cells from NOD mice selectively expand T regulatory cells independent of TCR activation

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Selective expansion of regulatory T cells (Tregs) is a highly desirable method for suppressing autoimmune responses and thus treating autoimmune diseases. Current approaches use T cell receptor (TCR) mediated signaling. However, the TCR dependent approaches are not suitable for clinical application as TCR mediated signaling can also cause expansion of pathogenic effector T-cells (Teff); thus reduce the ability of Tregs to control the disease, or worse can exacerbate the disease. Therefore, in this study we explored the possibility of expanding Tregs in the absence of TCR mediated signaling. We compared the phenotypes of Tregs expanded using TCR independent (using GM-CSF induced bone-marrow derived dendritic cells (G-BMDCs) or TCR dependent (using anti-CD3 antibody) approaches. Total CD4+ cells from non-obese diabetic (NOD) mice were co-cultured with either G-BMDCs or with splenic antigen presenting cells (APCs) supplemented with an anti-CD3 antibody. After 5 days, proliferating Tregs were examined by flow-cytometry for the expression of various Treg suppressive markers. When total CD4+ cells were co-cultured with G-BMDCs, we noted a significant and selective increase in the percent proliferating CD4+FOXP3+ Tregs but not CD4+FOXP3- effector T cells when compared to total CD4+ cells co-cultured with APCs and supplemented with anti-CD3. Additionally, of the CD4+FOXP3+ Tregs expanded in the presence of G-BMDCs a significantly higher percentage of proliferating cells were CD39+FOXP3+, a marker highly implicated in Treg suppressive capacity, when compared to those from cultures treated with anti-CD3. Furthermore, to unequivocally demonstrate TCR independent expansion of Tregs, G-BMDCs derived from MHC-/- mice were co-cultured with total CD4+ cells from wild type C57BL/B6J mice for five days. MHC-/- G-BMDCs also significantly increased the percentage of proliferating CD4+FOXP3+ cells. Of these CD4+FOXP3+ Tregs, a significantly higher percentage of proliferating cells were CD38+FOXP3+, another essential Treg suppressive marker, when compared to cells derived from cultures treated with an anti-CD3 antibody. These results suggested that G-BMDCs are capable of robustly and selectively expanding functional Tregs in a TCR independent manner, and thus may represent a more suitable approach for expanding Tregs for clinical application.

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Medicare's value-based reforms - are incentives enough?

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Encouraging physician practices to participate in value-based reforms is now a national priority. Following Medicare's Access and CHIP Reauthorization Act, providers will soon elect to join one of two value-based pathways: (1) the Alternative Payment Model program, where automatic 5% bonuses will be awarded to providers participating in accountable care organizations (ACOs) and other alternative delivery systems and (2) the new Merit-Based Incentive Payment System, which consolidates three existing reforms — Physician Quality Reporting System (PQRS), Physician Value-Based Payment Modifier, and EHR Meaningful Use Incentive Program — and adjusts payments according to performance on cost, quality, meaningful use, and clinical improvement activities. However, lackluster physician participation in these current initiatives highlights the importance of understanding the factors that lead to participation and success in these programs. Using data from a unique survey of 1,398 U.S. practices, we examined whether greater exposure to performance incentives, including both financial incentives and public reporting, was related to increased participation and improved performance in Medicare value-based reforms. Our study outcomes were ACO participation, Meaningful Use participation, and participation and quality in PQRS. We used multivariate regression analysis to examine practice-level relationships between prior exposure to performance incentives and participation and performance in Medicare's value-based reforms (computed as average marginal effects). We included interaction terms to assess whether these relationships vary across organizational characteristics and computed differences in marginal effects across 75th and 25th covariate percentiles ("delta marginal effect"). We found relatively low levels of performance incentives: fewer than half of practices (45.3%) participated in public reporting and only 0.43% and 0.22% of practices' income related to financial incentives for quality and efficiency, respectively. Practices with greater performance incentives demonstrated increased participation in Medicare ACOs (average marginal effect=0.033; SE=0.004), particularly those with greater use of health information technology (delta marginal effect=0.041; SE=0.014) and larger shares of patients with limited English (delta marginal effect= 0.016; SE=0.005). Performance incentives were unrelated to PQRS participation (average marginal effects=0.009; SE=0.016). Among PQRS participants, however, greater incentives were related to significantly worse quality (average marginal

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effect=-1.659; SE=0.706), particularly among practices with greater shares of Medicare revenue (delta marginal effect=-1.407; SE=0.398) and patients with limited English (delta marginal effect=-1.751; SE=0.868). Finally, incentives were not related to Meaningful Use participation (delta marginal effect=0.0141; SE=0.013). We conclude that increasing performance incentives and complementary efforts to improve technological infrastructure represent powerful levers to enhance participation in value-based reforms. At the same time, performance incentives alone appear insufficient to drive subsequent quality improvement among participants.

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Modeling electrophysiological interactions between mesenchymal stem cells and cardiomyocytes for improved cell delivery cardiotherapeutics

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Human mesenchymal stem cell (hMSC) delivery has demonstrated promise in preclinical and clinical trials for myocardial infarction therapy; however, broad acceptance of this approach is hindered by a limited understanding of the mechanisms by which hMSCs interact with human cardiomyocytes (hCMs). Mathematical modeling is a powerful tool that can simulate intercellular electrical coupling between hMSCs and hCMs. Therefore, to better understand the impact of hMSCs in cardiac therapies, three original electrophysiology models were developed to represent an empirical triad of hMSC families having distinct ion channel currents. The electrical interactions between hCMs and hMSCs were predicted by coupling the ten Tusscher models of human endocardial, midcardial, and epicardial hCMs to the hMSC models developed in this study. Significant pro-arrhythmic electrophysiological consequences were revealed at the cellular level for the two hMSC models expressing delayed rectifier-like human ether `a-go-go K+ channel 1 (hEAG1). Specifically, substantial decreases in action potential duration (APD) suggest hEAG1-expressing hMSCs may induce ventricular tachycardia and are capable of pro-arrhythmic electrical remodeling. The third family of hMSCs, absent of hEAG1 channel activity, demonstrated notably lower action potential variations, making it a preferred candidate for enhanced efficacy of hMSC cardiotherapeutics. A model parameter sensitivity analysis confirms these findings and supports the claim of larger APD variability caused by hEAG1-expressing hMSCs, underscoring the difficulty in predicting outcomes using such cell types. Electrophysiological complications can be exacerbated at the tissue level due to decreased conduction velocity following hMSC supplementation, independent of the hMSC family. In summary, this study provides novel electrophysiological models of hMSCs, demonstrates pro-arrhythmic potential of hMSCs when coupled to hCMs, and proposes that isolating a subset of hMSCs absent of hEAG1 channel activity may offer superior effectiveness and safety as a cell delivery cardiotherapy.

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Transcriptional Profiling Reveals an Aging Signature in the Mouse Lung that Persists During Influenza Infection

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Rationale: Aging is associated with increased morbidity and mortality attributable to influenza infection. In mice, infection with influenza A has been shown to alter the phenotype of alveolar type II (AT2) cells. We sought to determine whether an unbiased analysis of the transcriptional profiles of alveolar macrophages (AM) and AT2 cells could identify differences between young and old mice in the naïve state and in the presence of influenza infection. **Methods:** We FACSorted AM and AT2 cells from young (3 months) and old (18 months) C57BL/6 mice, both naïve and challenged with influenza A virus (WSN) (4 days post-inoculation). After RNA extraction and poly(A) enrichment libraries were constructed and single-end RNA-seq was performed on Illumina NextSeq 500 platform. Principal component analysis and K-mean clustering were performed and were used to guide functional enrichment analysis of the RNA-seq data. **Results:** We observed greater mortality with the same dose of influenza virus administered to mice aged 18 months compared with mice aged 3 months. Principal component analysis of the RNA-seq data demonstrated that 82.3% and 82.3% of the variance within the data in AM and AT2 cells correspondingly were explained by influenza infection and age. Influenza infection explained 60% and 61.9% of the variance in AM and AT2 cells, correspondingly, and was associated with upregulation of genes in characteristic pathways related to inflammation and response to stress. Age explained 23.2% or 20.4% of the variance in the data for AM and AT2 cells and separated the 16 AM and 16 AT2 samples into two distinct groups. Moreover, this age-dependent separation persisted in the presence of influenza infection. K-mean clustering revealed non-overlapping clusters of genes upregulated or downregulated specifically in response to influenza or aging. Functional analysis of genes downregulated in AT2 cells with age using GO annotation pathways identified enrichment for genes involved in extracellular matrix organization, cell adhesion, lung development, and anatomical structure morphogenesis, genes downregulated in AM were related to the cell cycle. **Conclusions:** Unbiased transcriptional profiling reveals an age-related signature in mouse AM and AT2 cells sufficient to distinguish young and old mice. This signature persists during influenza infection. The genes downregulated with age in AT2 cells are associated with processes related to lung structure and development, while genes downregulated in AM related to the cell cycle. Transcriptional profiling of individual cellular populations in the lung represents a promising tool to identify molecular changes that underlie the increased susceptibility of older mice to influenza A infection.

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Expression of MHCII on tumor cell surface enhances the immune response to triple negative breast cancer *in vivo*

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Anti-tumor adaptive immune responses are now being recognized to play a central role in the growth and progression of many types of cancer. Among these is the highly heterogeneous and aggressive triple negative breast cancer (TNBC), which lacks expression of the estrogen receptor, progesterone receptor, and Her2/neu. Our collaborators in the UAB Comprehensive Cancer Center (CCC) recently found that when TNBC tumor cells turned on several genes in the Major Histocompatibility Complex Class II (MHCII) pathway and expressed MHCII protein on the cell surface patients experienced greatly prolonged progression free survival (unpublished data). We therefore suspect that tumor cell surface expression of MHCII may stimulate a tumor-specific CD4+ T cell response. To test this hypothesis, we transfected TS/A cells, a murine metastatic breast cancer cell line, with the human MHCII Transcriptional Activator (hCIITA) gene under the control of a doxycycline (dox)-inducible promoter. We confirmed, both *in vitro* and *in vivo*, that the transfected cells expressed MHCII on the cell surface only after dox exposure. TS/A-hCIITA cells were then injected into the mammary fat pads of Balb/c mice and the immune response kinetics were assessed. When tumor-bearing mice were given dox via drinking water tumor growth was notably delayed relative to non-dox exposed controls. Analysis of excised tumors by flow cytometry revealed a significantly enhanced tumor-specific CD8+ T cell response within the tumor itself as well as the subcutaneous draining lymph node (sLN). This bolstered CD8+ response may indirectly suggest an enhanced contribution of CD4+ T helper cells. Interestingly, dox-exposed mice showed no difference in numbers of CD4+;CD25+;FoxP3+ regulatory T cells (Tregs) relative to controls in either the tumor tissue or sLN. Our findings, therefore, may suggest against Tregs as the most influential immune cell governing tumor outgrowth and rapid expansion in TNBC. While the precise contributions of each cell type are yet to be discerned, it seems likely that breast tumor cells expressing MHCII surface protein promote a tumor-specific immune response that delays tumor growth *in vivo*.

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Protein Kinase C-theta and Vimentin Modulate Multiple Facets of Regulatory T-cell Function

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Regulatory T-cells (Tregs) prevent autoimmune and alloimmune reactions, including graft-versus-host disease (GVHD). Two recent clinical trials demonstrated that in patients undergoing hematopoietic stem cell transplantation, adoptive transfer of Tregs significantly reduced GVHD severity, but did not eliminate disease. One potential way to augment Treg-mediated inhibition of GVHD is to increase Treg suppressive potency. Treg-specific inhibition of protein kinase C-theta (PKC-θ) enhances Treg function. However, it is unclear whether PKC-θ inhibition can boost Treg function in a systemic inflammatory condition like GVHD. Furthermore, the mechanism by which PKC-θ inhibition augments Treg function is unknown. Using a mouse model of GVHD, we found that freshly isolated Tregs treated with the clinically available PKC-θ inhibitor AEB071 suppressed GVHD mortality and severity significantly better than DMSO treated Tregs. As Tregs exert much of their protective effect against GVHD early in the course of the disease, we analyzed proliferation of GVHD-causing conventional T-cells (Tcon) on D4 after transplant. We observed a significant reduction in Tcon proliferation in mice given AEB071 treated Tregs compared to DMSO treated Tregs. We then performed multi-photon microscopy on D4 and found that compared to DMSO, AEB071 treated Tregs significantly increased Tcon velocity and displacement. This suggests that compared to DMSO, AEB071 treatment augments Treg suppression of Tcon priming. Mechanistically, AEB071 treatment augmented expression of the suppressive molecules Neuropilin-1 (Nrp1) and Lymphocyte activation gene 3 (Lag3) on Tregs. Antibody blockade of Nrp1 and Lag3 in transwell suppression assays reduced the effect of AEB071 treatment, suggesting that these may be functionally important after PKC-θ inhibition. PKC-θ inhibition also reduces phosphorylation of mTORC2 targets FoxO3a and Akt phospho-site S473, but not mTORC1 targets S6, 4E-BP1 or Akt phospho-site T308. This suggests reduced mTORC2 activity. As mTORC2 alters Treg metabolism, we investigated Treg metabolic profiles and found that AEB071 treatment significantly increased Treg Oxygen Consumption Rate (OCR) and uptake of fatty acids, both of which are associated with augmented Treg function. Phosphoproteomic analysis identified a significant reduction in the interaction between PKC-θ and the intermediate filament vimentin after AEB071 treatment, which was confirmed by confocal microscopy. Vimentin siRNA also disrupted PKC-θ/vimentin interactions and significantly increased Treg suppression *in vitro* compared to control Tregs. Vimentin siRNA treated Tregs also have augmented expression of Nrp1 and an increase in OCR. In summary, our data demonstrate that PKC-θ interacts with mTORC2 and vimentin to modulate multiple aspects of Treg function, and that PKC-θ inhibition may be a viable method to enhance the efficacy of Treg therapeutics.

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Oxidized CaMKII Causes Atrial Fibrillation Susceptibility in a Diabetic Mouse Model

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Background: Atrial fibrillation (AF) and diabetes mellitus (DM) are significant, unsolved public health problems; DM is a major risk factor for AF. Both AF and DM are associated with increased reactive oxygen species (ROS), suggesting a ROS responsive disease signal could be a mechanistic link between AF and DM. The multifunctional Ca2+ and calmodulin-dependent protein kinase-II (CaMKII) is activated by oxidation of paired methionines in the regulatory domain. Oxidized CaMKII (ox-CaMKII) is increased in atrial myocardium from diabetic patients and causes ryanodine receptor (RyR2) phosphorylation that promotes pathological intracellular Ca2+ release and Ca2+ triggered arrhythmias. We hypothesize that DM increases myocardial ox-CaMKII, RyR2 hyperphosphorylation and AF. **Methods and Results:** C57BL/6J mice with streptozocin-induced type 1 DM demonstrated increased AF susceptibility following atrial burst pacing protocol compared with citrate buffer-treated wild-type (WT) controls (70% vs. 25%, p = 0.01). Ox-CaMKII was increased in atrial tissue from diabetic mice compared to controls, consistent with a role for ox-CaMKII in this mouse model. Diabetic ox-CaMKII resistant knock-in (MM-VV) mice and diabetic mice with myocardium-restricted transgenic overexpression of methionine sulfoxide reductase A, a reductase that reverses ox-CaMKII, were protected from DM increased AF susceptibility with AF inducibility rates of 18% in each group ($p < 0.01$, compared with diabetic WT mice). Atrial myocytes from diabetic wild type mice demonstrated increased RyR2 mediated Ca2+ spark frequency, triggered membrane excitability and delayed intracellular Ca2+ decay compared to controls. Diabetic knock-in mice resistant to CaMKII-mediated RyR2 phosphorylation (S2814A) were protected from increased AF susceptibility, with AF rates of 22% ($p < 0.01$, compared with diabetic WT mice). All groups of streptozocin treated mice had similar plasma glucose increases. Our data suggest that ox-CaMKII transduces the pro-arrhythmic effect of hyperglycemia through RyR2. **Conclusions:** Hyperglycemia increases AF susceptibility and increased ox-CaMKII is associated with increased AF in this diabetic mouse model. Genetic manipulation of an ox-CaMKII pathway can protect against AF susceptibility in DM. These findings suggest that ox-CaMKII is a critical proarrhythmic signal in DM and may provide a therapeutic target for management of AF in diabetic patients.

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Urinary Synthetic Reporters to Detect Intact Cell-Surface Cancer Biomarkers

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Early detection of cancer is linked with improved patient outcomes, yet many cancer patients are first identified with advanced stage disease and require an invasive biopsy, and in the developing world the needed medical infrastructure is often cost-prohibitive. Thus, there is an urgent need for noninvasive, inexpensive, sensitive and specific cancer detection tools. Blood-based cancer biomarkers are unreliable indicators of disease due to low concentration and slow dynamics. Point-of-care assays relying on synthetic biomarkers filtered into the urine have shown promise in noninvasively detecting many diseases, including colorectal cancer. These methods exploit upregulated proteases in the local tumor environment by administering protease-sensitive nanoparticle (NP)-bound peptides into circulation; enzymatic peptide cutting releases reporters into the blood that are subsequently filtered into the urine and can then be detected by techniques such as enzyme-linked immunosorbent assay. Yet, this method is limited to detecting natural protease activity. We aim to broaden this technology to quantify aberrant receptor expression. We created bifunctional fusion proteins, composed of anti-receptor binders linked to exogenous enzymes, to pair receptor overexpression with an artificial local enzymatic upregulation that can be exploited by enzyme-sensitive NP-bound substrate-reporter peptides. Using epidermal growth factor receptor (EGFR) as a translatable model target, anti-EGFR fibronectin-based binders connected to exogenous proteases, such as tobacco etch virus protease (TEVP) or sortase, were shown to simultaneously preserve both binding and catalytic functionality against peptide substrates in a bound, cellular context. These results, as well as data evaluating how molecular architecture and administration plan drive modularity, activity and pharmacokinetic properties of the overall system in a mouse model, will be discussed. Further, as this system could benefit from gains in enzymatic activity and the sequence space of sortase and TEVP has been insufficiently investigated, we aimed to engineer sortase and TEVP variants with enhanced activity. The results of our enzyme engineering efforts, involving rationally-guided combinatorial libraries largely targeting the substrate-binding region and active site, will be discussed. We propose that the knowledge obtained from these studies regarding optimal design of a fusion protein/NP-substrate-reporter system, as well as optimal library design for enzyme engineering, will inform the implementation of the full urinary diagnostic platform, which will be tested in mice with variable tumor burden using receiver operating characteristic analysis to evaluate the performance and limits of the current technology. These current and future studies will optimize the urinary synthetic biomarker technology that could noninvasively and inexpensively diagnose aberrant receptor expression, such as EGFR overexpression, in a sensitive and specific manner.

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Monocyte-derived Alveolar Macrophages Contribute to the Development of Lung Fibrosis and Persist in the Lung Over the Lifespan

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Rationale: Idiopathic Pulmonary Fibrosis (IPF) is a progressive, age-related and lethal disease for which there are no effective therapies. Recently investigators have used lineage tracing techniques to show that tissue resident alveolar macrophages are long-lived self-renewing cells which persist over the lifespan with minimal input from the bone marrow. During injury monocytes are recruited to the lung where they differentiate into alveolar macrophages. **Hypothesis:** We sought to determine whether Caspase-8 plays a role in the differentiation of monocytes into alveolar macrophages during the development of lung fibrosis and whether these monocyte derived alveolar macrophages contribute to fibrosis severity. **Methods:** We treated two strains of mice with a specific deletion of Caspase 8 in monocytes, macrophages and dendritic cells in the lung, (LyzM-Cre/Caspase8fl/fl and CD11c-Cre/Caspase8fl/fl), two strains of triple transgenic mice with a macrophage specific deletion of Caspase-8 in a RIPK3 null background (CD11c-Cre/Casp8flox/flox/RIPK3-/- and LyzM-Cre/Casp8flox/flox/RIPK3-/-) and their wild-type controls (Caspase8fl/fl and RIPK3-/-) intratracheally with either bleomycin (0.025IU/mouse) or an adenovirus encoding an active form of TGF-β (1x108 pfu/mouse). We measured mortality and the severity of lung fibrosis using standard techniques. In whole lung digests, we quantified inflammatory cell populations and measured the expression of polarization markers using flow cytometry. Targeted (QuantiGene 2.0) and broad (RNA-seq) transcriptomic profiling were used to determine gene expression profiles of FACS-sorted macrophage populations. **Results:** During the development of fibrosis, monocytes recruited into the lung from the bone marrow transition sequentially to differentiate into interstitial macrophages and then alveolar macrophages. These monocyte-derived alveolar macrophages are nearly indistinguishable from tissue-resident alveolar macrophages by flow cytometry, however; using lineage tracing and transcriptome (RNA-seq) profiling we found that tissue-resident and monocyte-derived alveolar macrophages respond differently during the lung fibrosis. Monocyte-derived alveolar macrophages persist in the lung for months after the initial insult. Mice, deficient for caspase-8 in macrophages failed to differentiate from interstitial macrophages to profibrotic monocyte-derived alveolar macrophages. Competitive bone marrow chimera experiments confirmed the importance of Caspase-8 in macrophage differentiation. Simultaneous deletion of Caspase-8 and RIPK3 rescued fibrosis and restored population of monocyte-derived alveolar macrophages. **Conclusions:** We discovered a novel role for Caspase-8 in macrophage differentiation. Our data identify monocyte-derived alveolar macrophages as drivers of pulmonary fibrosis and suggest tissue resident alveolar macrophages contribute little to the development of fibrosis. Monocyte-derived alveolar macrophages persist in the lung long after bleomycin induced injury, providing a possible mechanism to explain the increased susceptibility to lung fibrosis during aging.

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Macrophages Contribute to Hypertension Development by Impairing the Pre-junctional Alpha-2 Autoreceptor on Sympathetic Neurons

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Hypertension is associated with increased sympathetic nerve activity and inflammation. Vascular sympathetic outflow is mediated by Ca2+ dependent release of norepinephrine (NE) onto arteries, resulting in vasoconstriction and blood pressure elevation. The pre-junctional alpha-2 autoreceptor (a2-AR) regulates the extent of NE release by inactivating nerve terminal N-type Ca2+ channels, thus, decreasing intracellular accumulation of Ca2+. We hypothesized that in hypertension, macrophages disrupt a2-AR mediated inhibition of N-type Ca2+ channels in vascular-projecting sympathetic neurons. **Methods:** Hypertension in rats was achieved by DOCA-salt treatment. Immunohistochemistry was used to assess macrophage infiltration. Vascular-projecting sympathetic neurons were identified by *in vivo* retrograde labeling of mesenteric arteries using cholera-toxin Alexa Fluor 594. Whole cell patch clamp was used on cultured sympathetic neurons to evaluate Ca2+ current inhibition following activation of a2-AR. Liposome-encapsulated clodronate (LEC) was used to deplete animals of macrophages. **Results:** Macrophage infiltration increases in the mesenteric vasculature of hypertensive rats. Ca2+ current inhibition via a2-AR activation is impaired in hypertensive rats. Dialysis of GppNHp (non-hydrolyzable GTP analog) inhibits Ca2+ current equally in normotensive and hypertensive rats. LEC treatment in DOCA-salt treated rats prevents vascular accumulation of macrophages, reduces the extent of hypertension, and preserves a2-AR mediated inhibition of Ca2+ current. **Conclusions:** Hypertension is associated with macrophage infiltration and macrophage-associated impairment of a2-AR mediated inhibition of Ca2+ current in sympathetic neurons. These effects are independent of factors downstream of a2-AR activation.

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Parameters for abolishing conditioned place preference for cocaine from running and environmental enrichment in male C57BL/6J mice

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Recent evidence suggests that four weeks of voluntary wheel running can abolish conditioned place preference (CPP) for cocaine in male C57BL/6J mice, but key parameters need to be worked out before the mechanism behind the observed

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behavioral effects of running can be understood. The primary objective of this study was to determine the duration and timing of exposure to running wheels necessary to reduce CPP, and the extent to which the running per se influences CPP as compared to environmental enrichment without running. Insights gained into what parameters constitute effective interventions for drug-to-context associations that form the basis of the CPP paradigm will be helpful to individuals trying to achieve complete abstinence from drug use. A total of 239 male mice were conditioned for 4 days twice daily with cocaine (10 mg/kg) and were then split into 7 different intervention groups prior to 4 consecutive days of CPP testing. The short sedentary group (SS; n=20) were housed in normal cages for 1 week. The short running group (SR; n=20) were housed with running wheels for 1 week. The short running group followed by a sedentary period (SRS; n=20) were housed with running wheels for 1 week and then normal cages for 3 weeks. The sedentary group followed by a short running period (SSR; n=20) were housed in normal cages for 3 weeks then 1 week with running wheels. The long sedentary group (LS; n=66) were housed in normal cages for 4 weeks. The long running (LR; n=66) group were housed with running wheels for 4 weeks. The long environmental enrichment group (EE; n=27 were placed in cages with multiple novel toys that were rotated on a weekly basis for 4 weeks. Levels of running were similar in all running animals. Both running and environmental enrichment reduced CPP relative to sedentary groups; however, running tended to produce a greater reduction of CPP. One week of wheel running was sufficient, and it did not matter whether the running was preceded or followed by a 3 week sedentary period. Results suggest the abolishment of cocaine CPP from running is robust , occurring with as low as 1 week of intervention, and that both increased physical activity and enrichment likely contribute to the phenomenon.

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DNA methyltransferases demonstrate reduced activity against arabinosylcytosine: Implications for epigenetic instability in AML

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Emerging cancer treatments are deeply rooted in mechanism, achieving targeted and rational inhibition of pathways driving tumorigenesis. This paradigm marks a deviation from early strategies in cancer treatment, where anti-metabolites were identified and found to have clinical efficacy without the prerequisite for insight into their mechanisms of action. One notable example of this earlier paradigm is arabinosylcytosine (araC), which has been a mainstay in the treatment of Acute Myeloid Leukemia (AML) since the 1970s. The recent recognition of epigenetic instability and altered DNA methylation in AML demonstrates the importance of elucidating the impact of araC treatment on DNA methylation. Here, we address this question using *in vitro* approaches, interrogating the intrinsic reactivity of DNA methyltransferases (MTases) against araC incorporated into synthetic, DNA substrates. First, using a restriction endonuclease-based assay, we qualitatively demonstrate that bacterial and human DNA methyltransferases preferentially methylate deoxycytidine (dC) as compared to araC. To quantify the extent of discrimination, we employed a radioisotope labeling-based assay and show that while the de novo methyltransferase DNMT3A/3L shows a 3-fold discrimination, the maintenance enzyme DNMT1 exhibits greater than 100-fold activity against dC as compared to araC. These observations offer support for a mechanistic model in which araC treatment promotes passive demethylation at sites of araC incorporation into DNA, thereby offering a putative mechanism by which treatment with araC might itself contribute to epigenetic instability and disease relapse in AML.

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Non-metabolized food volumization using polytetrafluoroethylene for therapeutic weight loss

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Satiety is largely influenced by gastric distention and therefore an increase in ingested food volume yields increased satiety. A novel weight loss technology has been prototyped which is an inert food volumizer that is non-metabolized thus contributing no caloric content to the diet yet increasing satiety and subsequently reducing eating. Polytetrafluoroethylene (PTFE) in powder form is proposed as an ideal material for this application since it is inert, resistant to stomach acid, smooth (will not scratch gut lining), heat resistant (can be cooked with food), precedented in medical applications, generally regarded as safe, and will not be absorbed (with proper particle size design). Previous animal trials have demonstrated safety and efficacy of PTFE ingestion in rats via 90-day feeding trials and histopathological examination showed no hepatic effects. A clinical trial is undergoing IRB review and commercialization is in preparation.

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L3MBTL2 modulates DNA double strand break repair and is a novel therapeutic target for breast cancer

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Background: DNA damaging agents induce double strand breaks (DSBs) and are used extensively in cancer therapy. Yet, resistance is often seen due to efficient sensing and repair of DSBs by the DNA damage response pathway. Understanding DNA repair mechanisms would help reveal new methods to overcome resistance. Here, we studied the role of Lethal (3)

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malignant brain tumor like protein 2 (L3MBTL2) in DNA repair. Since L3MBTL2 is differentially expressed in normal vs. cancer cells, we believe it to be an excellent therapeutic target. We hypothesized that loss of L3MBTL2 would induce a defect in DSB repair and augment cytotoxicity with DNA damaging agents. **Methods:** DNA damage, DNA repair capacities, cell survival, cell cycle distribution, and protein levels and interactions were assessed. **Results:** Loss of L3MBTL2 increased γH2AX foci, a marker of DSB by 4 fold. This coincided with 90% reduction in homologous recombination and non-homologous end joining mediated DSB repair capacities, independent of cell cycle or transcriptional effects. Interestingly, the mechanism involved modulation of ubiquitin signaling. Importantly, loss of L3MBTL2 markedly sensitized cancer cells to radiation and PARP inhibition. **Conclusions:** L3MBTL2 plays a role in DSB repair. Loss of L3MBTL2 is synthetic lethal with PARP inhibition and sensitizes cells to radiation. L3MBTL2 may be a novel target to overcome therapeutic resistance in sporadic cancers.

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BBS3 plays a unique role the pathogenesis of fetal hydrocephalus in a ciliopathy mouse model

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Introduction: Hydrocephalus is a neurodevelopmental disorder that affects up to 3/1000 births and is a significant cause of perinatal and pediatric morbidity and mortality despite surgical treatments. Primary cilia play an important role in CNS development. This is exemplified in Bardet-Biedl Syndrome (BBS), an autosomal recessive, heterogeneous, pleiotropic human ciliopathy in which ventriculomegaly occurs at a higher incidence than in the general population. We use a BBS mouse model to explore molecular mechanisms underlying hydrocephalus with the goal of facilitating pharmacologic treatment. We hypothesize that abnormal apoptosis in periventricular regions leads to an obstructive form of congenital hydrocephalus in the BBS3 knockout (KO) mouse. **Methods:** We compared BBS3 KO mice to littermate controls and other BBS knockout mice. We characterized disease onset and progression by quantifying ventricular and total brain areas of H&E-stained sections from embryonic day 17 (E17) to birth (P0) ($n \geq 3$ per group and genotype). We performed Evans blue dye studies in P0 and adult mice ($n \geq 5$ per group and genotype) to determine whether the hydrocephalus is communicating or obstructive. To examine whether impaired apoptosis of neural progenitor cells is involved in the pathophysiology, we performed TUNEL assays in periventricular regions early in the development of hydrocephalus ($n \geq 5$ per group and genotype). Finally, we measured CSF electrolytes, glucose, and osmolality for adult mice ($n \geq 5$ per group and genotype). **Results:** Ventriculomegaly in BBS3 KO mice appears as early as E17 ($p<0.05$, Student's t test). Impaired flow of Evans blue dye is seen in 60% of P0 BBS3 KO pups and 100% of adult BBS3 KO mice, consistent with non-communicating (obstructive) hydrocephalus. An approximately 50% decrease in apoptosis of cells in the cerebellar ventricular zone is seen in BBS3 KO compared to WT ($p<0.05$, one-way ANOVA with Tukey multiple comparison test). Adult CSF composition is the same between BBS3 KO and controls, although glucose is diminished in BBS3 KO animals ($p<0.001$, Student's t test). **Conclusion:** BBS3 KO mice develop fetal-onset hydrocephalus that progresses to a severe form of obstructive hydrocephalus. BBS3 KO mice display a significant reduction in apoptosis in periventricular regions relative to controls, suggesting an accumulation of neural progenitor cells. Further studies are needed to determine whether the accumulation of these cells underlies obstructive hydrocephalus in BBS3.

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Androgen exposure enhances urinary tract infection severity in a novel murine model

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Urinary tract infections (UTIs) occur predominantly in females but also affect substantial male patient populations; indeed, morbidity in complicated UTI is higher in men. Because of technical obstacles, preclinical modeling of UTI in male mice has been limited. We devised a mini-surgical bladder inoculation technique that yields reproducible upper and lower UTI in both male and female mice, enabling studies of sex differences in these infections. Acute uropathogenic Escherichia coli (UPEC) cystitis in males of two mouse strains recapitulated the intracellular bacterial community pathway previously shown in females. However, surgically infected C3H/HeN females exhibited more robust bladder cytokine responses and more efficient UPEC control than males. Compared with females, C3H/HeN males displayed a striking predilection for chronic cystitis. Further, C3H/HeN males developed more severe pyelonephritis, as well as 100% penetrant renal abscess (a complication that is rare in female mice). Antecedent gonadectomy sharply abrogated these phenotypes in male mice, while ovariectomy had no effect on the development of chronic cystitis in C3H/HeN females. Administration of exogenous testosterone in C3H/HeN females or castrated males induced severe pyelonephritis and abscess formation. Collectively, these data suggest that susceptibility to severe UTI is strongly influenced by androgen exposure in both males and females, while estrogen has no appreciable influence on the development of chronic cystitis. Susceptibility to severe UTI could be likewise induced with 5-α-dihydrotestosterone or abrogated via treatment with the antiandrogen flutamide, demonstrating that specific activation of the androgen receptor potentiates severe UTI. These data substantiate the long-standing presumption that anatomic differences in urogenital anatomy confer protection from UTI in men; however, as observed clinically, male sex was associated with more severe UTI once these traditional anatomic barriers were bypassed. Further, our findings suggest that modulation of androgens may represent a potential therapeutic route to combat recalcitrant or recurrent UTI.

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Targeting Senescent Cells as Therapy for Type II Diabetes: Studies in Diet-Induced Obese Mice

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In obesity, as in aging, senescent cells accumulate in multiple tissues including adipose tissue. Cellular senescence is an essentially irreversible growth arrest that occurs when cells undergo significant stress due to telomere shortening, oncogene activation, or metabolic insults. The accumulation of senescent cells, as well as their senescence-associated secretory phenotype (SASP), has been linked to tissue dysfunction and is thought to be a driver of age-related chronic disease. We have found that senescent cells accumulate in adipose tissue of diet-induced obese (DIO) mice, and that clearance of these senescent cells, either by genetic means or by novel senolytic therapy, ameliorates metabolic phenotypes. For example, mice that have undergone senescent cell clearance or treatment with senolytics exhibit improvements in glucose and insulin tolerance testing, and have lower glycosylated hemoglobin (HbA1c%). In addition, we see an increase in the extra- to intra-peritoneal adipose ratio following chronic senescent cell clearance or senolytic therapy. It appears that clearance of senescent cells also leads to enhanced adipose progenitor cell function and a reduction in mean adipocyte size in subcutaneous adipose. These results suggest that senescent cells play a role in metabolic phenotypes associated with high fat diet, and that clearance of senescent cells, or ablation of the SASP, may be an interesting therapeutic target in obesity and diabetes.

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Mouse thymic virus (MTV) is a murine betaherpesvirus closely related to HHV6/7

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Over fifty years ago, a natural murine pathogen, called the mouse thymic virus (MTV) or murid herpesvirus 3, was shown to cause severe thymic necrosis in neonatal mice, characterized by a near complete loss of CD4+ T-cells (Rowe and Capps, J Exp Med, 1960). However, the identity of this virus and its relationships to other viruses as well as the mechanisms by which it induced the underlying pathology remained incompletely understood. We sought to address these issues by determining the complete genome sequence for MTV which allowed further analysis. After verifying that an inoculum of our viral stock showed the unique biological characteristics of MTV, including causing gross thymic necrosis and loss of CD4+ T-cells, we inoculated a BALB/c pup on the day of birth. DNA was harvested directly from the thymus at 7 days post-infection and libraries were generated for both Pacific Biosciences sequencing as well as Illumina MiSeq sequencing. Mouse genomic data were eliminated by aligning sequencing reads to the mouse genome sequence. The long-read PacBio sequencing data and short-read Illumina data were independently assembled and contig sequences were compared to obtain the full genome sequence of MTV. We assembled a single contiguous, unambiguous 156Kb genome for MTV consisting of 114 predicted open reading frames (ORFs). Each ORF was individually subjected to comparative analysis using the BLASTP algorithm against the non-redundant database of protein sequences curated by the National Center for Biotechnology Information. Many ORFs were most closely related to genes found in roseoloviruses and the structure of the MTV genome was similar to HHV6/7. We determined the genetic relatedness of MTV to other mouse and human herpesviruses by generating a phylogenetic tree comparing the catalytic domain of DNA polymerase amongst various herpesviruses. Our analysis showed that MTV is a betaherpesvirus more closely resembling HHV6A, HHV6B, and HHV7 than another murine betaherpesvirus, mouse cytomegalovirus. Genome copy number quantification revealed that MTV replicated in the thymus but not in other tissues. Collectively, these data strongly suggest that MTV is a mouse homolog of HHV6/7. Supporting the sequence relationships, the human roseoloviruses are known to infect T cells and studies on humanized mice show that intrathymic infections with HHV6 lead to a decrease in human CD4+ T-cells similar to mice during MTV infection. Since MTV leads to near complete CD4+ T-cell depletion in mice, profound T-cell loss may also occur in HHV6/7-infected humans.

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Identification of SLE-associated risk variants in the STAT1-STAT4 locus and their effect on differential Transcription Factor Binding

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Systemic Lupus Erythematosus (SLE or lupus) is a chronic autoimmune disease with debilitating inflammation that affects multiple organ systems. The STAT1-STAT4 locus is one of the first and most highly replicated genetic loci associated with SLE risk. In this study, we aimed to identify all the SLE-associated common variants at this locus most likely to be causal and to further identify the biological mechanism mediating the increased disease risk. We genotyped 328 SNPs spanning the STAT1-STAT4 locus in 13,581 subjects representing four ancestral groups. We performed imputation and applied frequentist and Bayesian statistical analyses to identify the individual variants statistically most likely to causally increase lupus risk. We further used a separate larger African-American study to generate an Ancestry Informed Credible Set (AICS) of four variants. We

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computationally predicted differential transcription factor (TF) binding of AICS variants and identified the AT-hook family of TFs as a strong candidate for three of the four AICS variants. After identifying AT-hook family member HMGA1 as binding to rs11889341 through DNA Affinity Precipitation Assay (DAPA) followed by mass spectrometry, we confirmed binding of HMGA1 to two of four AICS variants with genotype-dependent binding by DAPA and Electrophoretic Mobility Shift Assay. In summary, we used large genetic datasets to identify a set of variants that are most likely to be causal for the STAT1-STAT4 association with increased lupus risk and identified a potential disease-risk mechanism in which HMGA1 differentially binds three genetic variants in a lupus-risk haplotype. *SLEGEN Collaborators: Dr. Marta E. Alarcon-Riquelme, Dr. Lindsey A. Criswell, Dr. Patrick M. Gaffney, Dr. Chaim O. Jacob, Dr. Robert P. Kimberly, Dr. Carl D. Langefeld, Dr. Kathy Moser Sivils, Dr. Betty P. Tsao, Dr. Timothy J. Vyse

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Small Molecule Inflammatory Inhibitors Prevent the Early Development of High Fat Diet-Induced Non-alcoholic Fatty Liver Disease in Male C57BL/6J Mice

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Nonalcoholic fatty liver disease (NAFLD) is the hepatic manifestation of both metabolic and inflammatory diseases, and it has become pervasive worldwide. Inflammation, including inflammation resulting from free fatty acid (FFA) activation of toll-like receptor (TLR) signaling, has been suggested to be an essential component of the pathophysiology of NAFLD. High fat diets (HFDs) promote an increased uptake and storage of FFAs and triglycerides (TGs) in hepatocytes, which initiates steatosis and induces lipotoxicity and inflammation. The objectives of this study were to evaluate the efficacy of novel small molecule inhibitors of inflammation developed in our laboratory, including a TLR inhibitor [phenylmethimazole (C10)], to delay and/or prevent HFD-induced steatosis in a C57BL/6J mouse model of high fat diet (HFD)-induced NAFLD. The mice were fed a HFD (60 % fat, 20% protein, 70% carbohydrate) and divided into 5 groups (N=5 for each group): sham injection (stress control), DMSO (vehicle control), phenylmethimazole (C10), COB-187, and COB-214. Each compound was administered once daily at a dosage of 1mg/kg for 6 weeks. Histological examination of liver sections from mice in this study using hematoxylin and eosin (H&E) and Oil Red O staining revealed less hepatic lipid accumulation in mice treated with HFD-fed COB-187 and COB-214 when compared to mice in the HFD-fed DMSO group. Hepatic triglyceride quantification analysis revealed that liver tissues of mice treated with HFD-fed C10, COB-187, and COB-214 had less triglyceride than mice in the HFD-fed DMSO and sham control groups. COB-214 had the greatest inhibitory activity. Differential gene expression of key mediators of lipogenesis were determined by real-time PCR. Specifically, the mRNA level of acetyl-coA carboxylase (Acc) was significantly elevated in the HFD-fed sham, DMSO, and C10 groups when compared to the HFD-fed COB-187, and COB-214 groups. Future directions for this study include, immunohistochemistry and real-Time PCR to detect the presence and location of inflammatory cells (lymphocytes and macrophages) in the liver and to determine changes in hepatic expression of pro-inflammatory cytokines and chemokines (IL-6, TNFα, MCP-1). Moreover, an extended study will be conducted to determine the efficacy of the compounds to prevent or delay the development of nonalcoholic fatty liver disease at several additional time points (0, 6, 12, and 16 weeks). At the conclusion of this study, we hope to have established the efficacy of a novel class of small molecule inhibitors of inflammation for the treatment/prevention of NAFLD.

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Cigarette Smoke Exposed Ferret as a Novel Model for Chronic Obstructive Pulmonary Disease

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Chronic Obstructive Pulmonary Disease (COPD) is the third leading cause of death in the United States and there are no specific therapies to combat disease progression. COPD includes both emphysema and chronic bronchitis and is characterized by airway mucus obstruction that is associated with accelerated loss of lung function and mortality. Recently our lab has characterized a novel sub-phenotype of COPD that resembles Cystic Fibrosis (CF), with patients exhibiting reduced CFTR activity, enhanced mucus production, and pronounced impairment of mucocilary clearance. To establish the impact of acquired CFTR dysfunction, my lab has developed a cigarette exposure system for ferret, which is an animal model that we show to recapitulate the aforementioned pathologies of COPD seen in humans. We are interested in determining whether chronic bronchitis can be pharmacologically ameliorated by activating CFTR and we hypothesize that augmenting CFTR function will improve mucocilary clearance and reverse manifestations of chronic bronchitis in ferret. Results could establish CFTR as a viable therapeutic target in chronic bronchitis and confirm the role of CFTR dysfunction as an underlying mechanism of the disease.

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Differential Effects of Radiotherapy on Distinct Bone Compartments

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Post-radiotherapy (RTx) bone fragility fractures are a frequent complication in radiation oncology. Previous work using a mouse hindlimb irradiation model demonstrated reduced trabecular bone, increased cortical bone, and decreased strength in

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the distal femur post-RTx. Furthermore, a drastic increase in osteoclast number and destruction of trabecular bone 2-4 weeks post-RTx was observed. Using the same limited field hindlimb irradiation model, we tested the hypothesis that irradiation would reduce the quantity of bone differently in distinct compartments of the femur. Female BALB/cJ mice aged 12 weeks were subjected to unilateral hindlimb irradiation in four consecutive daily doses of 5 Gy. Contralateral femurs served as non-irradiated controls. Mice were euthanized four days prior to the first radiation dose, and at 0, 1, 2, 4, 8, 12, and 26 weeks post-RTx, where 0 is the first day post-RTx (n = 9–13). Whole femurs were assessed by micro-computed tomography. Paired t-tests were performed at each time point, with significance accepted at $p < 0.05$. The four individual bone compartments examined in this study were Diaphyseal Cortical (DC), Metaphyseal Cortical (MC), Metaphyseal Trabecular (MTb), and Epiphyseal Trabecular (ETb). By 4 weeks post-RTx, DC quantity decreased, while MC quantity increased, as evidenced by their respective cross sectional areas and mean thicknesses. The ratio of MTb bone to total volume (BV/TV) had a biphasic response, increasing from weeks 0-2 and then decreasing below control levels from week 4 onward, despite a small but consistent elevation in bone mineral density (BMD). By 26 weeks, few trabeculae remained. ETb bone had decreased BV/TV at weeks 2, 4, and 26, normalizing between 4 and 26 weeks; however, its BMD was elevated from weeks 8-26. Both ETb and MTb showed decreased connectivity past 4 weeks. Whole measures of the distal 1/3rd of the femur (containing MC, MTb, and ETb bone) showed an increase in BV from 0-12 weeks, returning to normal by 26 weeks, while BMD decreased transiently at 1 week and was later increased at 8 and 12 weeks, returning to normal by 26 weeks. By 26 weeks, DC and MTb bone were decreased, while MC bone was increased and ETb bone showed mild changes. The loss of mechanically stabilizing trabeculae combined with loss of mid-shaft thickness may synergize to greatly increase risk of fracture in the femur post-RTx. The differential effects observed indicate that while studies focusing on single bone compartments can be useful, it is necessary to simultaneously investigate multiple compartments of bone to fully understand changes post-RTx.

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Auditory System Dysfunction in a Model of Blast-induced Traumatic Brain Injury

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Blast injuries to the outer ear have been well-documented, but more recent investigations have revealed blast-induced traumatic brain injuries (bTBI) may also damage the central auditory nervous system (CANS). In addition to a shift in hearing threshold, people exposed to blasts encounter difficulties in speech detection and sound recognition in the background of noise, deficits which can impact activities of daily living. Sounds are also often used in post-bTBI mental health treatment such as cognitive behavioral therapy which has been demonstrated to be less effective for brain-injured individuals. Unfortunately mild bTBI is difficult to diagnose in the early post-injury phases and, as a result, treatment is often delayed until after chronic symptoms are firmly established. As the CANS is a well-studied neuronal pathway in animal models, investigating central auditory dysfunction after bTBI may shed new light on the time course and pathophysiology of post-bTBI changes in brain structure and function. An established mild bTBI rat model known to incite intracranial deformation, elevation in oxidative stress, and neuroinflammation was used. Rats exposed to similar level of blast-noise but not blast-pressure were used as sham animals. Distortion product otoacoustic emissions (DPOAEs) for cochlear function and three types of auditory evoked potentials: auditory brainstem responses (ABRs), envelope following responses (EFRs), and middle latency responses (MLRs) were assessed on Sprague Dawley rats (3-4 months) in three sessions: pre-blast (baseline), two weeks after blast, and four weeks after blast. Blast-exposed rats had: (1) Higher hearing thresholds for clicks and high frequency tones; (2) Decreased ABR evoked potentials in the auditory nerve, lateral lemniscus, and inferior colliculus for click and 8 kHz tone stimuli (3) Reduced EFR amplitude at low amplitude modulations (16-128 Hz); and (4) Smaller MLR amplitudes evoked by click or 8 kHz tone compared to shams. No major differences were observed in DPOAE cochlear input-output functions at low frequencies, but high frequency reductions in function were apparent. The results suggest frequency-dependent peripheral + central degradation of auditory processing occurs in the cochlea, auditory nerve, brainstem, midbrain, and thalamus, even for the relatively simple sound stimuli presented in the current experiments. Noninvasive measurements of auditory evoked potentials may provide a novel avenue for diagnosing mild bTBI in the days to weeks following blast exposure. Further work to investigate CANS neurophysiological changes in the context of bTBI mechanisms of injury and real-world sound stimuli is warranted to gain more fundamental understanding of the causes and implications for post-blast auditory system dysfunction.

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Nanodiamond-Gadolinium(III) Probes for Tracking Cancer Cell Growth *In Vivo* Using High Field Molecular Magnetic Resonance Imaging

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Magnetic Resonance Imaging (MRI) is considered the modality of choice for live imaging because of its ability to noninvasively attain three-dimensional images with high spatial resolution. In the context of cancer, the ability to noninvasively track cancer cells *in vivo* would allow researchers to study their distribution, development and metastatic potential in pre-clinical models. To visualize cells by MRI, they must be labeled with MRI contrast agents (CAs). T1-shortening gadolinium(III) [Gd(III)]-based CAs

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are easily synthesized, provide positive (bright) contrast, and have high clinical utility, but suffer from low sensitivity compared to T2-shortening iron oxide nanoparticles or optical and nuclear modalities. To address these limitations, we have developed nanodiamond-Gd(III) conjugates [ND-Gd(III)] that provide state-of-the-art T1-weighted contrast. Nanodiamonds (NDs) are uniform, 4-5 nm carbon-based nanoparticles wholly suited for bioagent delivery due to their scalability, biocompatibility, and easily modifiable surface. Aminated NDs were peptide-coupled to Gd(III) chelates bearing a six-carbon carboxylate linker to synthesize ND-Gd(III), affording Gd(III) loading of 1.6 ± 0.3 mmoles/g of ND. We then evaluated the ability of ND-Gd(III) to label MDA-MB-231 human breast cancer cells. ND-Gd(III) demonstrated a 300-fold increase in intracellular delivery of Gd(III) compared to clinical Gd(III) chelates. The labeling efficiency obtained using ND-Gd(III) is unprecedented for T1 CAs. We further demonstrated that as few as one hundred ND-Gd(III)-labeled cancer cells can be easily identified in a dense background of agarose using T1-weighted MRI. Finally, we show that ND-Gd(III)-labeled cancer cells can be xenografted into immunocompromised mice and corresponding tumor growth can be tracked by T1-weighted MRI up to 26 days *in vivo* without significant decrease in contrast-to-noise ratio.

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Tubby regulates lysosomal function through a neuroendocrine signaling axis

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Obesity has reached epidemic proportions globally, and is a major risk factor for chronic diseases such as type 2 diabetes, cardiovascular diseases and hypertension. However, the pathophysiological link tying these conditions together is not well understood. One of the few monogenic obesity syndromes in mammals results from mutations in the Tub gene, which codes for Tubby. Tub is expressed only in neurons of the central nervous system, with highest expression in the hypothalamus – a region of the brain known to regulate adiposity. Loss-of-function mutations in Tub present with neurosensory defects (blindness and deafness) and morbid peripheral obesity. The molecular basis of these findings remains to be unraveled. Caenorhabditis elegans provides a powerful model to investigate the Tubby signaling pathway and its roles in neuroendocrine regulation of fat metabolism, due to its genetic tractability, conserved metabolic pathways, and compact neuronal architecture. Mutations in the C. elegans Tub homolog tub-1 cause analogous phenotypes of neurosensory deficit and peripheral lipid accumulation. We identified that neuronal deficiency of tub-1 leads to peripheral lysosomal dysfunction in the fat storage tissue, which is not innervated. We thus hypothesize that tub-1 modulates fat metabolism through a neuroendocrine regulatory axis of lysosomal function. We sought to elucidate both upstream and downstream components of this novel axis. A candidate-based screen for tub-1 regulators revealed ser-5, a G protein-coupled receptor, as the upstream activator of tub-1 in neurons. We subsequently performed IP-mass spectrometry studies to uncover the TUB-1 interactome. This approach identified the G protein subunit gpc-1 as a putative transducer of the ser-5 signal to tub-1. Additionally, we discovered that a large proportion of neuropeptides are transcriptionally repressed in tub-1 mutant animals. In silico analysis revealed the transcription factor sptf-1 as a putative tub-1 dependent master regulator of neuropeptide synthesis. To test if this novel neuroendocrine axis is evolutionarily conserved, we tested Tubtub loss-of-function mutant mice for lysosomal defects. Preliminary studies on livers of Tubtub mice showed misregulation of lysosomal enzymes at both mRNA and protein levels. Live cell imaging by Stimulated Raman Scattering microscopy revealed hepatosteatosis and hepatomegaly. Further studies are underway to delineate the precise biochemical defect in lysosome function. Together, our findings reveal a novel neuroendocrine axis of lysosomal regulation by Tubby.

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Pragmatic sequencing of advanced breast cancers reveals a complex genetic landscape

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Introduction: Genetic heterogeneity is a hallmark of advanced cancers and a proposed mechanism by which tumors become resistant to treatment. Metastatic, heavily pretreated, and aggressive cancers are the most fatal and most difficult to treat. As such, discovering the genetic landscape of these cancers could reveal the genetic mechanisms of their behavior. However, many large cancer sequencing projects, e.g., The Cancer Genome Atlas (TCGA), have specimens that are predominantly from early stage, untreated, and non-metastatic tumors. We hypothesized that a pragmatically aggregated population of advanced breast cancers would present a genetic landscape enriched for genetic mechanisms of treatment resistance and indicative of aggressive phenotype, distinct from those in TCGA. **Methods:** In accordance with a Vanderbilt University IRB-approved protocol, targeted exome sequencing data from a cohort of 198 patients with breast cancer who underwent clinical molecular profiling was reviewed. Sequencing was performed on formalin fixed paraffin-embedded specimens according to specifications by the FoundationOne assay (Foundation Medicine, Cambridge MA). Data for 925 patients with breast cancer sequenced by TCGA was obtained using the cgsdr package in the R program for statistical computing (v3.2.2) which was also used for statistical analysis. All appropriate statistical tests were two-sided, and confidence intervals were calculated by 500-fold bootstrap. **Results:** In comparison to TCGA population, patients in the clinical cohort were younger (median 52 vs 58 years, p=6.5x10-7), had a more advanced stage (median IV vs II, p=2.2x10-16), and had higher mutational burden (median 7.41 vs. 2.77 mutations/megabase, p=2.2x10-7). In the clinical cohort, 98.2% of patients were stage IV versus 2.1% in TCGA. In the clinical cohort, 33%, 17% and 50% of

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samples were from metastatic, nodal, and primary sites versus 0.5%, 8.9%, and 90% for the TCGA population. Mutations were present in different frequencies in the clinical cohort compared to TCGA: e.g., estrogen receptor 1 (7.0% vs 0.11%), e-cadherin gene (9.6% vs 0.21%), TP53 (60.1% vs 31.3%). Discussion: Patients who undergo panel exome sequencing in the context of clinical care represent a pragmatic population with a different genetic landscape from those sequenced in TCGA. They are younger, but have more advanced disease and higher mutational burden. They have higher rates of mutations that are likely to convey resistance to therapy and lead to more aggressive phenotypes. The data from these patients could be valuable in discovering genetic mechanisms of treatment resistance and novel means of overcoming resistance. Aggregating clinical genetic testing data presents a pragmatic opportunity to identify clinically-important tumor mutations.

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Spontaneous Jak3 mutation in Jackson Nr1d1 mouse line blocks innate lymphoid cell development

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The inbred mouse strain C57BL/6J is frequently used by experimental biologists in part due to the large number of targeted gene knockouts on the genetic background, which facilitates enhanced phenotype-genotype inferences. However, besides null alleles found in C57BL/6J mice, knockout mouse lines may accrue additional coding mutations unrelated to the targeted gene during their creation, as well as spontaneous mutations during inbreeding, which can confound analysis. We find that the B6.Cg-Nr1d1<tm1Ven>/LazJ mouse line purchased from Jackson Laboratories harbors a spontaneous mutation generating a SCID phenotype, with a particular inability to generate antigen-independent professional cytokine-producing innate lymphoid cells (ILCs). Bone marrow chimeras show that the phenotype is cell-intrinsic. Pedigree analysis demonstrates that the mutation has autosomal recessive inheritance, segregates independently from Nr1d1 knockout alleles, and originally occurred at Jackson. Exome sequencing identifies a single basepair insertion in exon 14 of Jak3. This mutation causes a frameshift mutation, which abrogates the C-terminal kinase domain and thus blocks signaling of common gamma chain cytokines. Sanger sequencing between littermates discordant for the SCID phenotype confirms this mutation. Jak3 inhibitors are approved clinically for the treatment of moderate-to-severely active rheumatoid arthritis and are known to substantially reduce the frequency of circulating natural killer cells. Our data suggests that Jak3 inhibition may also impact other ILCs and, to some extent, underlie clinical efficacy.

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Benefits of high intensity aerobic interval training in comparison with resistance and combined training in older and younger adults: A randomized trial with sedentary control

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Background: Sedentary lifestyles and low fitness are common with age and increase risk for chronic disease and death. Age-related declines in skeletal muscle mitochondrial content and function contribute to decreased aerobic fitness, muscle mass and strength. The health benefits of exercise are indisputable however, training responses vary with training intensity, and some individuals have few or no gains in cardiorespiratory parameters. High-intensity aerobic interval training (HIIT) can promote robust adaptations, yet its efficacy against other training modalities is incompletely understood. **Methods:** Forty-five young (18-30 years) and 27 older (65-78 years) sedentary adults underwent 12 weeks of supervised HIIT (4x4 minutes at 90%, 3 d/wk), resistance training (RT; 4 d/wk), or combined training (CT; 30 minutes at 70% and about half of the RT protocol) after a 12-week sedentary period. Peak aerobic fitness (VO2 peak) was determined by graded exercise test. Resting muscle biopsies were collected from the vastus lateralis for mitochondrial respiration and total RNA sequencing. **Results:** VO2 peak was ~33% lower at baseline in older adults and increased ($p<0.05$) with HIIT (Y: +29%, O: +17%) and CT (Y: +14%, O: +19%). Muscle mitochondrial respiration was 24% lower in older adults at baseline then increased following HIIT (Y: +49%, O: +69%), but increased with CT only in young adults (Y: +38%). At baseline, 433 genes were altered with aging, representing pathways regulating mitochondrial and muscle growth. HIIT increased expression of 418 genes in young and 555 genes in older adults with predominant changes in muscle growth and mitochondrial pathways in older adults. RT increased 271 genes in young and 168 genes in older, predominantly from muscle growth pathways, while CT increased 301 genes in young and 91 genes in older adults. **Conclusions:** Older adults successfully performed HIIT with robust increases in aerobic fitness, mitochondrial respiration and gene expression of multiple pathways that are down regulated with age. Our results support HIIT as a stimulus for multiple metabolic pathways and may be incorporated into exercise prescriptions to minimize the number of exercise non-responders.

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SLX4IP regulates metastatic dormancy in breast cancer by controlling telomere length and stability

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In all subtypes of breast cancer (BC), metastasis is associated with therapeutic resistance and increased mortality. However, the cellular events that define metastasis remain poorly understood, and therapies targeting metastasis continue to be elusive. A critical stage in BC metastasis is dormancy, in which cells disseminate from the primary tumor and persist within the metastatic microenvironment in a state of quiescence for an indeterminate period of time. In the present study, we adopt a forward genetic approach to identify SLX4-interacting protein (Slx4ip) as a positive regulator of BC dormancy in the D2.OR murine breast carcinoma model of metastatic dormancy. SLX4IP is a member of the SLX4 structure-specific endonuclease (SSE) complex that functions in DNA damage repair and telomere homeostasis. shRNA-mediated knockdown of Slx4ip is sufficient to increase proliferative capacity, attenuate migratory behavior, and promote three-dimensional (3D) outgrowth of D2.OR cells *in vitro*, all of which are indicators of metastatic activity. Conversely, overexpression of Slx4ip in metastatically active D2.A1 cells results in opposing phenotypes. Moreover, mice injected with D2.OR cells exhibiting reduced Slx4ip expression show significantly increased pulmonary tumor burden, suggesting that loss of Slx4ip expression promotes tumor progression in a clinically relevant BC metastatic niche. Mechanistically, Slx4ip localizes at telomeres in our cellular models of metastasis, and loss of Slx4ip expression leads to an increase in telomerase expression and activity and a concomitant increase in telomere length. This increase in telomerase activity arises secondary to dysfunction of the alternative lengthening of telomeres (ALT) pathway, in which the SLX4 SSE has been implicated. This study provides a foundation for elucidating specific pathways that become aberrant as a result of telomere dysfunction in the metastatic microenvironment, as well as investigating the utility of the telomere maintenance machinery as a therapeutic target for metastatic disease.

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PAX8 leads to early progression of high-grade serous ovarian carcinoma through up-regulation of the FOXM1 pathway

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Ovarian cancer is the 5th leading cause of cancer mortality in women in the United States. High-grade serous carcinoma (HGSC) is the most common form of ovarian cancer and the most lethal. Part of the reason for this high mortality rate is lack of early detection and the heterogeneity of disease. It is crucial, therefore, for research to elucidate the early mechanistic changes that facilitate the development of ovarian cancer. Paired-box transcription factor 8 (PAX8) is a transcription factor expressed in cells of Müllerian origin, including the fallopian tube, that is essential for development. Though PAX8 expression may be acquired in adult human ovarian surface epithelium (OSE), Pax8 deletion does not impact ovarian development. In mouse OSE (MOSE), however, it is widely accepted that PAX8 is not expressed in the OSE. Recent research has shown models of HGSC derived from MOSE cells gain PAX8 expression. Amongst human HGSC clinical samples, PAX8 is expressed in ~80-96% of cells. In this study, we show that PAX8 increases proliferation and migration in MOSE cells through increased expression of several EMT factors such as N-Cadherin and Fibronectin. PAX8 is also known to regulate p53, which is mutated in 96-100% of HGSC cases and controls expression of FOXM1. The FOXM1 pathway is altered in ~70% of tumors and can be targeted with cell penetrating peptides. In this study we show PAX8 inhibited p53 activity and enhanced FOXM1 levels in MOSE cells. Targets downstream of FOXM1, such as BIRC5a were also up-regulated. In contrast, PAX8 knockdown in the mouse oviductal epithelium (MOE) cells did not affect the FOXM1 pathway and there was only a slight decrease in proliferation. There were no changes in migration, cell cycle, or apoptosis after PAX8 knockdown in MOE cells. Finally, despite an unknown cellular origin, most ovarian tumors and ovarian cancer cell lines express PAX8. Silencing PAX8 in these cancer cell lines resulted in apoptosis with a decrease in the anti-apoptotic protein BCL2. There was also a decrease in FOXM1 and its downstream proteins. The results presented here suggest that PAX8 has a role in the early mechanisms of ovarian cancer development and despite its unique action in oviductal and ovarian surface cells, its reduction in serous tumors provides a common mechanism for reducing cell survival.

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Extracellular Matrix Stiffness Regu… Endothelial Cell … …g of VEGF

WITHDRAWN

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Endothelial cell angiogenic processes are largely driven by vascular endothelial growth factor A (VEGF). The mechanical and biochemical microenvironment surrounding endothelial cells varies with disease progression. The conformation of fibronectin, a common ECM protein elevated with disease progression, can also be modified by biochemical (heparin) and mechanical signals (stretch or stiffness) revealing cryptic binding sites for growth factors such as VEGF. Mechanically, the stiffness of the extracellular matrix (ECM) can vary by over an order of magnitude in a young healthy artery to a stiffened aged vessel. We hypothesized that stiffening of the ECM disrupts VEGF-cell-matrix connections and resultant VEGF processing and signaling. We found VEGF interacted least with cell produced ECM on soft Fn-polyacrylamide gels, but the ability of these

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cells to internalize and produce downstream signals in response to VEGF was enhanced as compared to all other substrates. Modifying VEGF matrix binding by either blocking VEGF binding with sucrose octasulfate or enhancing VEGF binding with heparin respectively inhibited or enhanced VEGF cellular uptake. Cells bind to fibronectin protein through β1 integrin, which was found to have decreased activation in endothelial cells in stiff conditions. Blocking the activation of β1 integrin resulted in a decrease in endothelial cell VEGF internalization in stiff conditions that was not seen in the soft ECM conditions. Collectively, we showed soft ECM matrices enhance VEGF integrin-assisted matrix binding resulting in increased VEGF cellular uptake and downstream signaling. VEGF matrix binding is critical for VEGF receptor activation and signaling. These findings could be applied throughout multiple areas of vascular biology including the selective engineering of new vasculature or the creation of designer targets to inhibit angiogenesis. Ultimately, the results unfold new avenues for selectively inducing or attenuating VEGF signaling and subsequent angiogenesis.

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In vitro 3-Dimensional modeling of the tumor microenvironment in non-small cell lung carcinoma

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Lung cancer is the leading cause of cancer related death in the United States and worldwide. Five-year survival rates for non-small cell lung carcinoma patients have not improved in decades, and there are limited treatments for patients that are elderly or have advanced stage disease. Identification of therapeutic targets for advanced stage disease is essential for the development of novel therapies to halt the progression of this devastating disease. However, current 2-dimensional in-vitro models are not reflective of the tumor microenvironment and are inadequate in identifying these targets. In an effort to better understand lung cancer biology, we adapted a human lung fibroblast derived 3-dimensional matrix to study the interaction of lung carcinoma cells with the tumor microenvironment. We have demonstrated that carcinoma cells grown under these conditions display differences in cell morphology, proliferation, migration, and survival. Further, we can observe changes in the structure and substrate composition of the extracellular matrix (ECM). This allows manipulation of the matrix to better mimic the pro-fibrotic condition of the aging lung and also under stress of chemotherapy. We have shown that altering human fibroblast derived ECM enhances carcinoma cell growth. Additionally, carcinoma cell migration is enhanced at high cell density compared to low cell density on 3D matrix but not on 2D fibronectin-coated plastic. Thus, our 3D model demonstrates that we are able test ECM-to-cell interactions *in vitro* in a way that is not possible for 2D models. An understanding of how remodeling of the tumor microenvironment is involved in late stage disease will allow development of preventative and curative therapeutic strategies to halt tumor progression and metastasis.

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The Role of Centromeres in the Molecular Biology of Prostate Cancer

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The centromere is an essential component of the cellular machinery required for the faithful segregation of chromosomes during mitosis, a process that is significantly dysregulated in cancer. Though accurate centromeric assembly is vital for maintaining genomic stability and ensuring accurate cell division, the role centromeres play in cancer biology remains understudied. The highly repetitive underlying sequence that makes up the genomic landscape has made annotation of the centromeric region very difficult, leaving the study of centromere genomics and epigenomics as one of the last frontiers in the quest towards a more complete understanding of the human genome. New methodologies that can navigate the repetitive nature of centromeric sequences are thus required to effectively study centromere genetics and molecular biology in the context of cancer. We have developed novel bioinformatics tools that allow us to survey gene expression across large data sets to ascertain cancer specific expression of target genes. Our group has additionally developed a quantitative PCR assay to detect chromosome-specific centromeric sequences, with a focus on detecting α-satellite repeats, which are the hallmark of centromeres. Further, we have identified significant overexpression of the centromeric H3 histone variant CENPA in prostate cancer. Preliminary studies suggest that CENPA knockdown in prostate cancer cells limits their ability to proliferate. Centromeric α-satellite RNAs (CeASaRs) are known to modulate CENPA function, and we can demonstrate enrichment of specific CeASaRs when comparing prostate cancer to benign prostatic tissue. This enrichment was validated using our chromosome specific qPCR assay in whole cell RNA extracts from prostate cancer and prostatic epithelial cell lines. In view of the above, we hypothesize that centromeres represent a functionally important molecular signature that can drive prostate cancer biology.

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HMGN2 facilitates prolactin-induced transcription and promotes a tumorigenic phenotype by facilitating the loss of linker histone H1 from promoter DNA in breast cancer cells

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The polypeptide hormone prolactin (PRL) is essential for normal breast tissue growth and maturation; however, this hormone also contributes to breast cancer development. PRL binds to and activates the transmembrane PRL receptor (PRLr). The PRLr signals from the cell surface to the nucleus both by activating

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canonical signals, such as the Jak2/Stat5 pathway, and by directly translocating to the nucleus. We have previously shown that nuclear PRLr binds to the chromatin-modifying protein high-mobility group N2 (HMGN2). The PRLr recruits HMGN2 to the promoter of the PRL-responsive gene CISH (cytokine-inducible SH2-containing protein). At this promoter, HMGN2 stimulates transcription, but the mechanism by which HMGN2 does so is unknown. One potential mechanism previously identified is that HMGN2 binds to nucleosomes and induces chromatin decompaction by competition with chromatin-compacting proteins. Given this, we hypothesized that HMGN2 causes chromatin decompaction at promoters of PRL-responsive genes, allowing the transcriptional machinery to access the promoter DNA and initiate transcription. In these studies, the CISH promoter was examined by chromatin immunoprecipitation (ChIP) for factors regulating chromatin compaction, such as the linker histone H1.2. PRL stimulation resulted in the loss of histone H1.2 at the CISH promoter. Following HMGN2 knockdown, the loss of H1.2 was attenuated. Therefore, HMGN2 may stimulate CISH transcription by facilitating the loss of H1.2 from the promoter, likely through competitive binding. Consistent with this hypothesis, the decrease in CISH expression induced by HMGN2 knockdown was rescued by the additional knockdown of histone H1.2, further suggesting that the role of HMGN2 in CISH transcription involves the loss of H1.2. Consistent with transcriptional activation, PRL stimulation also resulted in increased RNA Polymerase II (Pol II) bound at the CISH promoter; knockdown of HMGN2 resulted in less bound Pol II. The nucleosome landscape at the CISH promoter was then mapped using the micrococcal nuclease (MNase) protection assay. Prior to PRL stimulation, the CISH promoter exhibited a bound nucleosome overlying the binding site of the necessary transcription factor Stat5a. PRL stimulation resulted in eviction of this nucleosome. At the cellular level, breast cancer cells expressing wild-type HMGN2 exhibited increased colony growth in soft agar compared to cells expressing mutant forms of HMGN2 in which either the nucleosome-binding domain or the chromatin-unfolding domain had been disrupted. These data suggest that HMGN2 stimulates PRL-induced transcription by facilitating the loss of histone H1.2 from the promoter, allowing Pol II to access the promoter DNA and drive the transcription of pro-proliferative genes, thus promoting a tumorigenic phenotype.

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Effects of preoperative chemoradiotherapy on tumor viability and patient outcomes in head and neck squamous cell carcinoma with cervical lymph node metastases

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Background: Head and neck squamous cell carcinoma (HNSCC) commonly metastasizes to cervical lymph nodes. Prior to surgical resection, patients may be treated with preoperative chemoradiation (pCRT) to decrease tumor burden. The effects of pCRT on histological tumor viability and patient outcomes have not been well studied. This study investigates HNSCC metastatic to cervical lymph nodes and correlates histologic and immunohistochemical (IHC) features to patient outcomes. **Methods:** 147 patients with HNSCC who underwent neck dissection at the University of Chicago were identified; 89 of these received pCRT. 47 patients (18 with pCRT and 29 without) had viable carcinoma in cervical lymph nodes, and 48 had only treatment effect (fibrosis, foamy histiocytes, nucleate or anucleate keratin debris). 52 patients (23 with pCRT and 29 without) had no sign of viable or treated tumor in cervical lymph nodes. IHC for markers of proliferation (Ki67, cyclin D1), apoptosis (caspase 3), and DNA damage (H2AX) were performed on lymph nodes with viable SCC or nucleate keratin debris. pCRT status and tumor viability were correlated to disease recurrence. **Results:** Of patients with viable SCC, those who received pCRT had an increased likelihood of recurrence or metastasis (72% vs 31%, p=0.005). Patients with viable SCC who received pCRT also had significantly lower expression of Ki67 (39% vs 24%, p=0.03) than those who did not, though expression of cyclin D1, caspase 3, and H2AX was not significantly different. Nucleate keratin debris had negligible levels of staining for all markers. Of the patients who received pCRT, those with viable SCC had increased rate of recurrence (72% vs 29%, p=0.001) compared to those with treatment effect only. Patients without any cervical lymph node SCC did not have significantly different rates of recurrence whether or not they received pCRT (43% vs 38%, p=0.69). Rates of recurrence between groups with viable SCC, treated SCC, and no signs of SCC, were not significantly different (49% vs 29% vs 33%, p=0.08-0.71). **Conclusion:** Patients who receive pCRT and have no viable cervical lymph node SCC remaining have superior outcomes to those with viable SCC. However, of patients with viable SCC, those who received pCRT had both worse outcomes and lower levels of Ki67 than those who did not. Viable tumor after CRT may be an indicator of tumor aggressivity. Nucleate keratin debris should not be considered viable carcinoma.

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Disruption of epithelial apical or basolateral polarity components causes hyperproliferation and disorganized collective cell migration

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The establishment and maintenance of apical-basal polarity is a defining characteristic and essential feature of functioning epithelia. Apical-basal polarity (ABP) proteins are also tumor suppressors that are targeted for disruption by oncogenic viruses and are commonly mutated in human carcinomas. Disruption of these ABP proteins is an early event in cancer development that results in increased proliferation and epithelial disorganization through means not fully characterized. First, using the

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Drosophila melanogaster wing disc epithelium we demonstrate that disruption of apical versus basolateral polarity results in increased proliferation via distinct downstream signaling pathways. Surprisingly, the Rho-Rok-Myosin contractility apparatus appears to play opposite roles in the regulation of this proliferative phenotype based on which polarity complex is disrupted. In contrast, non-autonomous TNF signaling appears to suppress the proliferation that results from apical-basal polarity disruption, regardless of which complex is disrupted. We also demonstrate that collective migration in the Drosophila ovarian follicle epithelium is disrupted in distinct ways depending on whether the apical or basolateral polarity complex is compromised. Using quantitative optical flow image analysis, we show that disruption of the apical polarity complex results in large holes in the migrating epithelial sheet while maintaining the coordinated movement of collective migration. On the other hand, following disruption of the basolateral complex, epithelial integrity is largely maintained, but the organization of collective motion decreases. These findings provide further insight into how disruption of epithelial apical or basolateral polarity complexes can alter cell proliferation and migration during early carcinoma development.

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Health and Fitness Status of Parent-Child Dyads: Single Sport Athletes Versus Multisport Athletes in the Competitive Adolescent Population

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Purpose: To determine if there is a difference in health status between single sport and multisport athletes in the competitive adolescent population. **Methods:** This cross-sectional study involved initial baseline surveys for competitive adolescent athletes and their parents (dyads), followed by fitness testing and accelerometer monitoring. The adolescents (ages 10-15) completed sports training history and injury history surveys; their parents completed musculoskeletal injury history and overall health status surveys. Athletes injured as a result of their sport also completed a survey reporting injury type, limitations and training patterns. Dyads also completed a 6 minute walk test, Bioelectrical Impedance Assay (BIA), blood pressure testing, and anthropomorphic testing. Both the parent and the adolescent were then given an accelerometer to wear for one week, and energy expenditures were measured. **Results:** Of the 19 dyads in the study, 12 completed anthropomorphic and physical activity measurements, while 7 dyad pairs completed only survey data. Single sport parents were more active than multisport parents with regards to 10-minute bouts MVPA (20.26 ± 28.4 vs. 0 ± 0, p=0.197) and 1-minute bouts of MVPA (30.3 ± 8.7 vs. 1.75 ± 1.25, p=0.103). Single sport children were more active than multisport children with regards to 5-minute bouts of MVPA (18.4 ± 14.6 vs. 3.4 ± 1.1, p=0.145) and 1-minute bouts of MVPA (24.1 ± 17.6 vs. 8.0 ± 4.5, p=0.191). Multisport parents are playing more than three times as much sports with their child compared to single sport parents (3.1 ± 1.1 vs. 0.82 ± .75, p=.0001). Single sport adolescents play more hours of competitive sports per week (p=0.1) and less recreational sports (sports for fun) per week (p=0.001) than their multisport peers. **Conclusion:** Our study found that more active parents (parents of single sport children) correlated with more active children (single sport children). These findings suggest that even in the competitive athlete population where ancillary factors like adolescent self-motivation might be in play, some degree of parental modeling is likely a correlate for PA. Our results also showed that multisport adolescents and their parents play significantly more weekly hours of sports together than single sport parent-child population. We postulate that this result will positively affect the health of the multisport adolescent population more than the single sport adolescent population, but a longitudinal study is needed to verify this claim.

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Intracellular Redox Active Metal Ions Mediate the Differential Susceptibility of NSCLC Cells to Pharmacological Ascorbate

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Despite recent advances in therapy, 5-year survival in patients with non-small cell lung cancer (NSCLC) and Glioblastoma Multiforme (GBM) remain at 16% and 4%, respectively. The use of pharmacological doses of ascorbate given intravenously has recently emerged as a non-toxic adjuvant to chemo-radiation, and has been demonstrated as a pro-drug for hydrogen peroxide (H2O2). However, the mechanism by which ascorbate selectively enhances sensitivity of cancer versus normal cells to chemo-radiation has yet to be fully elucidated. A growing body of evidence demonstrates increased steady-state levels of reactive oxygen species (ROS) and labile iron in cancer as compared to normal cells. Additionally, specific ROS, namely superoxide (O2•-) and H2O2, are capable of disrupting cellular iron metabolism. The current study tests the hypothesis that pharmacological ascorbate selectively enhances chemo-radiation therapy responses in NSCLC via a mechanism that involves increased redox-active metal ions in cancer cells. Our results demonstrate that pharmacological ascorbate is selectively toxic to NSCLC and GBM cells as compared to normal human bronchial epithelial cells (HBEpCs) and normal human astrocytes (NHAs), respectively. Importantly, ascorbate selectively sensitizes NSCLC and GBM cells, but not HBEpCs or NHAs, to chemo-radiation as measured by clonogenic cell survival assays. Furthermore, adjuvant ascorbate significantly increased overall survival in NSCLC and GBM murine xenografts as compared to chemoradiation alone. Ascorbate toxicity *in vitro* is inhibited by addition of exogenous catalase or iron chelators that inhibit metal ion redox-cycling. Furthermore, NSCLC patient tumor tissue demonstrated increased levels of O2•- and labile iron, suggesting that alterations in oxidative metabolism may underlie perturbations in iron metabolism resulting in the selective

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susceptibility of cancer cells to ascorbate. To more directly test this hypothesis, we utilized Crispr/Cas9 to delete mitochondrial superoxide dismutase 2 (SOD2) in HEL 92.1.7 cells. SOD2-KO cells demonstrated significantly increased labile iron, and importantly, susceptibility of ascorbate-mediated toxicity. Finally, there is evidence that ascorbate can increase the pool of labile iron. Indeed, the labile iron pool (LIP) of NSCLC and GBM cells significantly increased from baseline upon exposure to ascorbate as compared to HBEpCs and NHAs, and this was suppressed by overexpression of catalase. This body of *in vitro* and *in vivo* data support the hypothesis that increased ROS in cancer cells drives neoplastic changes in cellular iron metabolism, which results in selective sensitization of cancer vs. normal cells to pharmacological ascorbate. (Supported by ASTRO JF2014-1, The Carver RPOE in Redox Biology, T32CA078586, P30CA086862, T32GM007337, RO1CA166800, CA169046, and CA182804.)

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Airway Acidification Initiates Host Defense Abnormalities in Cystic Fibrosis Mice

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Defects in CFTR anion channels cause cystic fibrosis (CF). In humans and pigs, loss of CFTR impairs respiratory host defenses causing airway infection. But, CF mice are spared. In all three species, CFTR secreted HCO3- into airway surface liquid (ASL). In humans and pigs lacking CFTR, unchecked H+ secretion by the non-gastric H+/K+ ATPase (ATP12A) acidified ASL, which impaired airway host defenses. In contrast, mouse airways expressed little ATP12A and secreted minimal H+; consequently, CF and non-CF ASL had similar pH. Inhibiting ATP12A rescued host defense abnormalities in human and pig airways. Conversely, expressing ATP12A in CF mouse airways acidified ASL, impaired defenses, and increased airway bacteria. These findings help explain why CF mice are protected from infection and nominate ATP12A as a therapeutic target.

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In situ visualization of protein-protein interactions reveals molecular coordination required for dendrite morphogenesis

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Obtaining high-resolution information from a complex network while maintaining its natural context is a key challenge in biology. This is especially true of molecular networks, largely governed by protein-protein interactions, which underlie life's dynamic processes. Despite the invaluable information they have provided, genetic interaction screens and biochemical assays lack either evidence of direct protein association or an intact endogenous environment, respectively. In both cases, these static networks do not reveal the spatiotemporal context of interactions within a living system, limiting our ability to recreate or control processes at the cellular level This project seeks information critical to biological networks, the time and place of interactions between proteins within the brain of a living animal. Using optically transparent Drosophila embryos and Forster Resonance Energy Transfer as a proxy for protein-protein interaction, we expose interactions directly within their natural context through live imaging. We demonstrate that a ubiquitously present and developmentally essential molecular switch, Cdc42, physically associates with its signaling partners, WASp and Par-6, within the nervous system. We find that each partner, although broadly co-localized and thus capable of activating separate pathways, interacts with Cdc42 only at distinct times and places. Moreover, within individual neurons, this interactomic sub-network evolves spatiotemporally during dendrite morphogenesis, yet with a great deal of overlap - suggesting that these divergent signaling pathways coordinate to execute morphological change. Coupled with results from knockdown and constitutive activation experiments, our work demonstrates how restricted protein interactions play a decisive role in this developmental process. All together, our approach will provide unprecedented insight into how protein network dynamics give rise to complex cell behavior.

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Ependymal Cell Dystroglycan Regulates SVZ Neural Stem Cell Niche Development and Function

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The generation and maintenance of the brain requires the production of vast numbers of neural cells during development and maturity respectively. The largest population of proliferating cells in adult human, rodent, and primate brains is found in a unique neural stem cell (NSC) niche– the Subventricular Zone (SVZ). The SVZ generates neurons and glial cells at precisely the right time and place to support the generation of the brain, respond to injury, and meet the continuous need for new neural cells. Modulation of the extracellular matrix (ECM) by knocking out components such as laminin alters the production and maturation of cells that arise from the SVZ. Dystroglycan is a receptor found on cells in the SVZ that mediates their interaction with ECM proteins such as laminin. Our lab has characterized a major phenotype in neural-specific dystroglycan-knockout mice marked by significant disorganization and dysregulation of neural cells that make up and arise from the SVZ stem cell niche. We now report that removal of dystroglycan in the developing postnatal brain leads to aberrant notch signaling in the SVZ, altering the relationship between developing neural stem cells and their niche. Removing dystroglycan specifically in multicilliated niche support cells known as ependymal cells leads to disruption of ventricular zone ECM organization. Furthermore,

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the proper functioning of this dystroglycan-ECM axis is required for neural stem cell regulation, and genetic disruption of ependymal cell dystroglycan alters neural stem cell homeostasis. These defects may underlie observed cognitive and structural brain deficits in a subset of muscular dystrophies in which dystroglycan function is disrupted.

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Poly(ethylene glycol) hydrogels to promote *in vitro* salivary gland morphogenesis from primary salivary gland cells

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Permanent damage to the salivary gland due to radiation therapy for head and neck cancers results in chronic dry mouth (xerostomia), for which no regenerative treatment exists. Direct injection of submandibular gland (SMG) cells into damaged mouse glands has shown some regeneration and functional improvements, but with variable regeneration of acinar cells. To address these challenges, we have utilized poly(ethylene glycol) (PEG) hydrogels to encapsulate SMG multicellular aggregates (spheres), resulting in sustained viability *in vitro*. We sought to characterize hydrogel-encapsulated cell populations and enhance regeneration by promoting acinar cell survival and proliferation *in vitro*. Encapsulated SMG spheres show a ~3.5-fold increase in cellular ATP over 14 days, which together with numerous EdU+ cells, supports cellular proliferation. Fluorescent cell lineage tracing of both acinar and duct cells shows a decrease in the percentage of acinar and an increase in duct cell phenotypes. However, the percentage of acinar cells remained stable from day 7 to 14 post-encapsulation and the percentage of EdU+ cells co-labeled with the acinar cell marker increased from ~20% at day 1 to ~40% at day 14, indicating increased acinar cell proliferation at later time pointd. Based upon the role of duct cells as intermediaries during gland development, we speculate that expansion of duct cells during the initial stages of encapsulation is required for acinar cell survival and proliferation, possibly through matrix deposition. Current experiments are focused on incorporation of matrix cues such as laminin and cell-degradable functionalities to decrease initial losses in acinar cell survivability.

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Identification and Characterization of the Novel Cby-Interacting and BAR Domain-Containing Proteins FAM^{92}A and FAM^{92}B in Ciliogenesis

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Cilia are small hair-like projections extending from nearly all eukaryotic cell surfaces. Cilia are associated with critical cellular functions such as cellular motility as well as cell signaling and sensory functions. In recognition of this, intense research to elucidate the molecular mechanism of ciliary formation and maintenance has been undertaken in order to understand the many and heterogeneous diseases (cilia-related diseases termed the ciliopathies) resulting from ciliary defects. Chibby (Cby) is an evolutionarily conserved coiled-coil protein that was initially isolated as an antagonist of the canonical Wnt/β-catenin signaling pathway. Generation of CbyKO mice revealed phenotypes characteristic of ciliopathic diseases. Subsequently, it was revealed that Cby is a critical mediator of cilia formation through its ability to recruit membranous vesicles to the ciliary base. However, the molecular mechanism as to how exactly Cby facilitates recruitment and subsequent fusion of small vesicles to centrioles remains unclear. To further probe the molecular mechanism for Cby function in the fusion of small vesicles, we compiled a comprehensive list of potential Cby interacting partners, with a focus on basal body proteins. Tandem affinity purification (TAP) technology was used to identify the novel Cby interacting partners FAM92A and FAM92B. Beyond the few studies that suggest that FAM92A plays a role in embryogenesis, little else is known about the function of the FAM92 family of proteins. Members of this family contain a putative BAR-domain, a highly conserved domain that forms a crescent-shaped homodimer and is found in many proteins involved in membrane dynamics. We characterized FAM92A and FAM92B as novel Cby interacting partners that localized to the base of cilia. The BAR-domain of FAM92A and FAM92B was sufficient for this interaction as well as the FAM92 proteins ability to homodimerize. In the absence of Cby, FAM92A and FAM92B localization to the base of cilia was disrupted. This suggests that Cby acts upstream of the FAM92 proteins, possibly recruiting the FAM92 proteins to the basal body. Additionally, siRNA mediated knockdown of FAM92A decreased cilia formation, which implicates the FAM92 proteins involvement in cilia formation. Furthermore, ectopic expression of FAM92 and Cby induced membrane tubule-like structures. Overall, we have identified and characterized the BAR-domain containing proteins, FAM92A and FAM292B, as novel Cby interacting partners. The BAR-domain properties of FAM92 proteins could provide a crucial link between the ability of Cby to recruit vesicles and the subsequent membrane fusion necessary for cilia formation.

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Interdisciplinary Intervention to Decrease Emergency Department Utilization by Sickle Cell Disease Superutilizers

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Adults with sickle cell disease (SCD) have a high rate of acute healthcare utilization in the emergency department (ED), with the most common chief complaint at presentation being pain crisis. A relatively small subpopulation of SCD patients termed "superutilizers" (SU's) present to the ED at even higher rates and use a disproportionate amount of healthcare resources. In

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fact, previous independent studies have converged to find that about 15-20% of the SCD population were responsible for over 50% of the total ED visits of SCD patients. Taken together, this contributes significantly to both national healthcare expenditure and hospital overcrowding. Elevated healthcare utilization of SU's is partly attributable to previous findings that SU's, when compared with low-utilizing SCD patients, have been reported to be more severely ill (by clinical lab values and increased comorbidity prevalence), with higher subjective pain scale ratings and poorer quality of life. Moreover, SCD patients at large face many healthcare disparities, including physician-patient mistrust and delayed pain management. Previous studies have employed protocols to successfully address the burden posed by SCD patients, but there is severely limited data on SU populations. To address the healthcare burden of SU populations within our institution, with preliminary input from a community of sickle cell advocates and an interdisciplinary medical team, we developed and implemented the following protocol for adult SCD patients who "superutilize" ED and hospital resources. A comprehensive and continuous care plan in the form of a "medical home" and multidisciplinary team was established for these patients. In this protocol, we established a best practice advisory system, which provides a compact summary of patient-specific treatment information and recommendations from their specialty care team within the electronic medical record. An ED protocol that included standing orders was implemented to expedite the administration of analgesics and to reduce redundant blood draws and imaging. Finally, we provided education to the patients regarding outpatient clinic usage. When pre- and post-implementation periods of our study were compared, we found clinically important results, including: reduction of total ED visits; decreased hospital admissions; decreased total hours spent undergoing treatment in the ED and hospital; decreased left without being seen visits; and increased outpatient clinic usage. An important limitation of our findings is the small sample size of the study (n=10), though a clinically significant effect size was still observed. The results presented herein have significant implications for reducing national healthcare expenditure, increasing hospital throughput, and improving quality of patient care in a disadvantaged group.

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Expression and function of astrocytic connexin 43 in a model of pediatric traumatic brain injury

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Pediatric traumatic brain injury (TBI) is an important cause of morbidity and mortality in children, contributing to 80% of trauma deaths. Toddlers show the highest injury rates, with a >50% increase in hospitalizations between 2007 and 2010. Current treatment options are limited to supportive care and surgery, based on adult TBI guidelines. Adult TBI, however, is not an adequate model of pediatric TBI considering the vast developmental changes occurring in immature brains. There is a need to develop models of pediatric TBI that will define short- and long-term injury mechanisms in an effort to discover novel therapeutic targets. Evidence suggests the astrocytic protein connexin-43 (Cx43) is involved in TBI pathophysiology. Cx43 is a transmembrane protein that comprises hemichannels, facilitating extracellular communication, and functional gap junctions, hemichannel dimers facilitating communication between astrocytes. This study aimed to elucidate the role of Cx43 in a rat model of pediatric TBI by 1) characterizing Cx43 expression in the immature brain and 2) determining changes in Cx43 expression following TBI. For developmental studies, we used cortical tissue from postnatal day 0-100 (P0-100) wild-type rats. For TBI studies, we simulated diffuse pediatric TBI via weight-drop injury of P18-22 rats. Tissues were analyzed for mRNA and protein changes, and astrocyte-specific glial fibrillary acidic protein (GFAP) was used to assess injury severity. We found that Cx43 mRNA levels remain constant throughout development, while Cx43 protein levels are low initially and begin to increase after P14. In TBI experiments, we found no significant change in Cx43 mRNA or protein expression in mildly injured animals compared to uninjured animals. These results suggest 1) post-transcriptional regulation of Cx43 during development and 2) Cx43 expression does not change following mild diffuse TBI. Future studies should study Cx43 expression and function following severe TBI.

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Using multi-dimensional flow cytometry to characterize polyfunctional and cross-reactive T cell responses against naturally occurring mutant HCV antigen and hepatocellular carcinoma

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While adoptive cell transfer (ACT) has been gaining clinical success, this methodology offers potential to address biologic questions concerning a T cell's response against tumor or viral antigen. Using a high affinity, HLA-A2-restricted HCV NS3:1406-1415-reactive TCR, we have characterized cross-reactive recognition of wildtype and various naturally occurring mutant epitopes. Rather than using one dominant pro-inflammatory cytokine to characterize reactivity, which is the standard in the field, we characterized polyfunctional potential on a per-cell basis by simultaneously analyzing 7 different parameters, including CD107a, IFNγ, TNFα, IL-2, IL-4, IL-17A, and IL-22 using flow cytometry. Out of the 128 possible combinations of polyfunctionality, multivariate analysis in Pestle/SPICE yielded distinct "cytokine signatures" among CD4+ or CD8+ transduced T cells, often differing between donor PBL. This complex analysis also highlighted unique populations of T cells simultaneously producing both type 1 and type 2 cytokines that would not otherwise be identified using standard reactivity methods. Interestingly, while cytokine patterns differed among mutant epitope stimulation, these changes didn't necessarily correlate with TCR-pMHC KD or t1/2 measurements, suggesting

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affinity is not the only important factor governing recognition. Furthermore, while CD8 is necessary for certain mutant recognition, both affinity- enhancing and lck-binding signaling components are required. Additionally, comparing peptide vs. tumor stimulations highlighted a preferential loss of higher-level polyfunctional cells when stimulated by hepatocellular carcinoma cells engineered to express naturally processed NS3 and its variants. These multi-dimensional analyses emphasize the vast heterogeneity of T cells in a given culture or between donors as well as phenotypes that cross traditional boundaries set to "type" a T cell, suggesting the field has oversimplified T cell function. Further comprehensive analysis of T cells' polyfunctional potential will help shed light on T cells' biologic capacity and what may be the "best" T cell to use for ACT.

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Microbiota metabolites regulate host T cell differentiation: a tale of two Indoles

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The commensal microbiota is composed of greater than 1014 microbial cells, with the GI tract component of this population being the most dense and linked to host health both locally, as well as systemically. The underlying mechanisms used by the gut microbiota to regulate host health are largely undetermined, but once elucidated, they could provide new therapeutic approaches for a diverse collection of diseases linked to the microbiota such as inflammatory bowel disease, multiple sclerosis, cancer and asthma. Previous work from our lab identified eleven strictly microbiota-derived tryptophan (Trp) metabolites that modulate host cell aryl hydrocarbon receptor (AhR) signaling. We investigated the hypothesis that microbiota-derived Trp metabolites regulate host T cell function through the AhR as a microbiota-mediated mechanism to instruct host immune response towards a pro- or anti-inflammatory fate. Of the Trp metabolites, two—Indole and 5-HydroxyIndole—had the most robust and contrasting effects on T cell differentiation. Specifically, Indole augmented anti-inflammatory Treg differentation and reciprocally inhibited inflammatory Th17 T cell differentiation. In contrast, 5-OH-Indole promoted inflammatory effector T cell differentiation (Th1 and Th17) while conversely blocking Treg differentiation. Although both indole and 5-HI are AhR ligands, we found that Indole's effects on T cells required AhR signaling, whereas 5-OH-Indole was AhR-independent. These data suggest additional unidentified signaling pathways are affected by microbiota-derived Trp metabolites. In addition to *in vitro* characterization of these metabolites effects on cell signaling and differentiation, we verified that the effects of these two metabolites are also present in an *in vivo* system of T cell expansion and activation. Importantly, Indole and 5-HI also influence human T cell differentiation *in vitro* similarly to what was seen with mouse T cells. Taken together, these results suggest that the microbiota Trp metabolite signature could function as a mechanistic rheostat linking microbiota composition and host health

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Processing of paired stimuli in medial temporal lobe subregions perirhinal cortex, lateral entorhinal cortex, dentate gyrus and CA1 supports formation of a time-bridging associative response

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Acquisition of trace eyeblink conditioning (EBC) engages the hippocampus, however the single-neuron responses of perirhinal cortex (PR), lateral entorhinal cortex (latEC) and dentate gyrus (DG) have not been described during this task. Rabbits were trained on trace EBC (or given unpaired stimuli; Pseudo) and single-neuron activity was recorded in PR, latEC, DG and CA1 during learning and following a one-month consolidation period. A total of 2891 well-isolated neurons were recorded in PR (N=433), latEC (N=431), dorsal DG (N=1309) and dorsal CA1 (N=718). PR neurons responded to both CS (whisker vibration) and US (corneal airpuff), but showed little conditioning-specific activity. There was however an increase in significantly modulated PR neurons in conditioned animals, consistent with PR as a multimodal coincidence detector that may increase local LTP and transmission to latEC. LatEC and DG showed conditioning-specific trace-period responses starting before behavioral criterion. The magnitude of the stimulus response was reversed between EC responding maximally to CS, and DG firing maximally to US. LatEC showed trace conditioning-induced firing in high-firing rate cells that have not been described during spatial tasks, possibly reflecting local mGluR activation and learning-induced AHP decrease. Thus pre-criterion trace-bridging activity develops in latEC and DG with distinct stimulus-response profiles during temporal learning. PR, latEC and DG neurons showed similar response profiles on CR and no-CR trials, indicating that they are involved in learning associations but not in controlling the behavioral responses--as opposed to regions such as medial prefrontal cortex, whose neuronal activity differs between CR and no-CR trials. However, the trace-period response in rate-decreasing cells of CA1 is stronger on CR- than no-CR trials. While DG maintained an elevated number of responsive neurons post-consolidation, the characteristic firing rate profiles of EC and DG were diminished post-consolidation, consistent with an acquisition-specific role for these regions. The present study found that within medial temporal lobe, neuronal signals representing paired stimuli are transformed from individual stimuli in PR, to stimuli plus trace-bridging activity in EC and DG, to a behavior-linked response in CA1.

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Tumor necrosis factor and CD28 signaling play unique but complementary roles in the systemic recruitment of innate immune cells after Staphylococcus aureus enterotoxin A inhalation

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Gram-negative bacteria and lipopolysaccharide have been the focus of the sepsis research field for many years; however, Gram-positive bacteria, and particularly Staphylococcus aureus (S. aureus), have been found in septic patients at similar or greater frequencies. Among the different virulence factors contributing to the severity of S. aureus-induced infections, S. aureus enterotoxins have been known to cause a life-threatening systemic inflammatory response. Yet, how enterotoxins spread systemically and trigger the inflammatory cascade involving both innate and adaptive immunity is unclear. Here we showed in mice that after inhalation, S. aureus enterotoxin A (SEA) rapidly entered the bloodstream and activated T cells to orchestrate a systemic recruitment of inflammatory monocytes and neutrophils to blood and lymphoid tissues. To study the mechanism used by SEA-specific T cells that mediate this process, a systems approach revealed inducible and non-inducible pathways as potential targets. It was found that while tumor necrosis factor (TNF) induced neutrophil migration to blood, CD28 signaling, but not TNF, was responsible for the chemotaxis of inflammatory monocytes to blood and lymph nodes. However, both mediators triggered local recruitment of neutrophils into lymph nodes. Thus, our findings revealed a dual mechanism of monocyte and neutrophil recruitment by SEA-specific T cells relying on overlapping and non-overlapping roles for the non-inducible costimulatory receptor CD28 and the inflammatory cytokine TNF. The failure of anti-TNF therapies to ameliorate disease outcomes in septic patients could be due to reduced neutrophil count in blood leading to increased bacteremia. Therefore, during sepsis, there might be clinical value in inhibiting CD28 signaling to decrease T cell-mediated inflammation and recruitment of innate cells while retaining bioactive TNF to foster neutrophil circulation.

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Generation of antibodies against chromgranin A mimotopes in the context of MHCII class II

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BDC2.5 T cells are used in mouse models of type 1 diabetes, as their transfer results in rapid diabetes pathogenesis. Chromogranin A has been shown to be the natural ligand of BDC2.5 T cells as well as an autoantigen in human studies of type 1 diabetes. However, the exact role of this autoantigen is unknown due to lack of reagents. Two peptide mimotopes, p31 and p63, share a common amino acid motif with a natural cleavage product of Chromogranin A, WE14. We developed a novel antibody against mimotope peptide in the context of IAg7 to block BDC2.5 T cell activation. This antibody provides a new tool for discovery as well as possibility for antigenic specific therapies for type 1 diabetes.

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Interrogating neural circuitry underlying neuroeconomic decision-making in mouse models of addiction: A functional approach to translation

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Deliberative decision-making in humans comprises a number of capabilities: integrating sensory inputs, assessing motivational states, imagining and evaluating possible consequences, identifying potential costs versus gains, and executing coordinated action sequences. New theories see neuropsychiatric disorders like addiction as a consequence of vulnerabilities or failure modes in such decision-making processes. Current translational paradigms are based on creating models of disease in animals – thus measuring, for example, the effects of cocaine in self-administering rodents. Such approaches ask how drugs of abuse affect animal behavior and look for mechanistic explanations for those changes, which are then looked for in human populations. My work instead poses a functional approach to translation where I ask what the neural mechanisms are in humans and whether changing the functionality of those circuits in animals will produce the same aberrant behavioral effects. Since a number of brain structures are required to communicate intricately to produce such calculated behaviors during deliberative decision-making, a functional approach to translation is based on matching the underlying dysfunction in specific neural circuitry driving distinct aspects of decision-making information processing across species. For example, a human addict might be less willing to wait for a reward than a non-addicted individual. Instead of asking whether addicted rodents are also less willing to wait for a reward, I ask what are the neurophysiological changes that underlie that impulsivity in human drug-dependent users, and how can we impose those neurophysiological changes in rodents. If those neurophysiological changes also drive impulsivity in rodents, then I can ask how to treat those neurophysiological changes (to return rodent impulsivity back to normal). These treatments are more likely to translate back to humans. However, investigating information processing mediated in neural circuits in real-time during decision-making has been difficult to study partly because animal models to study cognition have been slow to evolve in approximating the complexity of human decision-making information processing and partly because advanced circuit investigation tools have only recently become available in mice. Thus, I developed a novel behavioral paradigm based on human neuroeconomic approaches in order to study decision-making in mice, with access to cutting-edge optogenetic interventions enabling

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interrogation of circuits with precise genetic, spatial, and temporal resolution. Further, my work is part of a larger collaboration translating the very same task I use in mice into neuropsychiatric patient populations coupled with functional magnetic resonance imaging. It is through this work my efforts aim to identify failure modes in neural circuitry underlying distinct aspects of deliberative decision-making.

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SWELL1 links adipocyte volume with insulin signaling

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Adipocytes have a remarkable ability to enlarge, increasing in volume >30X in the setting of obesity. Recent studies have highlighted a connection between adipocyte size, membrane tension and adipogenesis, suggesting adipocyte-autonomous stretch-sensitive mechanisms of lipid homeostasis. To date, no molecular candidates for this adipocyte-membrane stretch sensor have been proposed. Ion channels are membrane proteins that can signal in response to membrane-stretch, and accordingly provide plausible candidates for this undiscovered stretch-sensor. To examine this, we applied the patch-clamp technique to freshly isolated, mature mouse and human adipocytes and identified a novel stretch/swell-activated ionic current with characteristics of the Volume-Regulated Anion Current. We use selective pharmacology, and both shRNA-mediated and CRISPR/cas9-mediated silencing to show that SWELL1 (LRRC8a) encodes this stretch/swell current in primary and cultured adipocytes. We find that SWELL1 silencing reduces total intracellular lipid content in both primary and cultured adipocytes, likely by selectively preventing the activation of insulin-PI3K-AKT2 pathway. *In vivo*, we find SWELL1 expression is significantly induced in obese mice and humans compared to lean counterparts, suggesting a role in adipocyte hypertrophy. These results suggest that SWELL1 represents a novel stretch-sensor connecting cytoplasmic volume regulation with insulin mediated adipocyte growth pathways, particularly in the setting of obesity.

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Aortic Root Aneurysm in TASK Potassium Channel Mutant Mice

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Aortic aneurysms are a significant cause of morbidity and mortality. This persistent disease burden highlights significant gaps in our understanding of the genetics underlying aneurysm formation in aortic tissue. TWIK-related acid-sensitive K+ (TASK) channels are present in aortic tissue, however their function in the aorta has not been investigated. Prior work has suggested that TASK-1 and TASK-3 modulate vascular tone and neurohormonal regulation. Whether TASK-1 and TASK3 modulate aortic aneurysm formation is not known To address whether TASK channels are involved in aortic aneurysm development, we measured aortic root diameters by echocardiography in genetic TASK knockout mice. Aortic root size was measured serially in TASK-1/3 DKO [n=9], TASK-1 KO [n=24], TASK-3 KO [n=51], and wild type [n=34] mice every 4-8 weeks from 8 to 36 weeks of age. At a baseline of 8-12 weeks of age, wild type and TASK-1/3 DKO mice had similar aortic root measurements (1.69 mm and 1.77 mm, respectively). However, by 24-28 weeks, the TASK-1/3 DKO mice had significantly dilated aortic roots ($p<0.01$). At 32-36 weeks of age, the aortic root measured 1.99 mm in TASK-1/3 DKO mice and 1.78 mm in wild type mice ($p <0.01$; $p <0.01$ by repeated measures ANOVA). No difference in aortic root size was noted between TASK-1 KO, TASK-3 KO, and wild type mice. In preliminary experiments, histologic analysis of aortic root cross-sections showed a 28% increase in aortic wall thickness in TASK-1/3 DKO mice compared to wild type. Additionally, elastin fragmentation was observed on VVG stain in TASK-1/3 DKO mice. Aortic wall thickness for TASK-1 KO and TASK-3 KO mice were 13% and 12% greater than wildtype, respectively. However, elastin appeared normal on VVG stain for TASK-1 and TASK-3 KO mice. Based on prior work suggesting that TASK channels influence neurohormonal signaling, ongoing experiments are testing whether activation of angiotensin II type 1 receptors and their downstream signaling (ERK, SMAD2/3, p-38, and JNK) are altered in the aortic roots of TASK mutant mice. In summary, loss of both TASK-1 and TASK-3 channels leads to aneurysm of the aortic root with a thickened wall and abnormal elastin architecture. While loss of TASK-1 or TASK-3 channels independently lead to mild aortic wall thickening, both mutations are needed to observe aortic root aneurysm and elastin fragmentation.

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Transcriptomic Response of an *In Vitro* Tribological Model of Cartilage Surface Damage

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Introduction: Current models of injury-induced osteoarthritis apply a single, high-energy impaction to investigate disease progression, neglecting additional complex joint movement that occurs following an injury as well as other low-energy stresses at play. We developed a test sequence to induce superficial zone damage through shear stress caused by mechanical articulation against polyethylene, which has been clinically documented in causing surface injury. We evaluated this superficial zone damage model through cartilage gene expression response to the effect of varying culture period and the presence/ absence of shear stress. Methods: Bovine cartilage was randomized: control, articulation against polyethylene, or articulation

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against cartilage. Samples underwent a 1-day or 5-day pre-culture. Tribological testing used a joint motion simulator at physiological conditions, utilizing a moving contact point that preserves biphasic lubrication. Samples were loaded to 40N (~2MPa) and articulated for three hours for one day. Tissue was examined for viability and with RT-PCR for the following: GAPDH, aggrecan, caveolin-1, type II collagen, type III collagen, cartilage oligomeric matrix protein, hyaluronan synthase 2, and proteoglycan-4. Results: Culture period: Total mRNA decreased due to culture with significant decreases detected at 5d pre-culture ($p \leq 0.01$). Following 1d pre-culture ACAN and HAS2 mRNA were significantly decreased compared to freshly procured samples ($p \leq 0.05$). However, after 5d pre-culture, mRNA abundance for all genes assayed, except CAV1, was significantly decreased. Articulation: Viability was decreased in PE damaged samples. PE articulation increased surface damage but no changes in chondrocyte morphology. PRG4 for the PE group was the only gene/ group to express a significant difference between the wear area and peripheral area (1d & 5d: $p \leq 0.05$). Four genes (COL3A1, COMP, HAS2, PRG4) showed significant fold changes and patterns within and across groups. Discussion: This investigated the mechanobiological response of healthy/ damaged cartilage with a unique damage model via shear stress. First, pre-culture did not influence viability or histological appearance; however, a 5d period decreases in mRNA abundance for matrix genes. Second, testing created an altered profile for several genes of interest. A sequence of the shear-induced damage model presented followed by articulation against cartilage would allow a better *in vitro* representation of *in vivo* activity, when an individual experiences damage to their articular surface (damage model) and continues with their daily living (continued loading and surface motion). The model would allow investigation into mechanisms of cartilage repair and into therapies for cartilage regeneration. Biomarkers could be identified and monitored to track progression of cartilage damage as it is influenced by mechanical and biological factors.

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Dysregulation of microRNA-146b/Fbxl10 Governs ASC Behavior Under Obese Stress

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Obesity, defined by the pathologic expansion of visceral and subcutaneous adipose tissue, is central to the development of cardiovascular disease and metabolic syndrome. Resident adipose tissue stem cells, termed adipose tissue derived stem cells (ASCs), mediate the lifelong turnover of adipocytes and are thus crucial to adipose tissue homeostasis. Unfortunately, the effects of and mechanisms through which obesity alters ASC physiology, mediating adipocyte dysfunction and subsequent obese pathologies, are poorly characterized. We reveal dysfunction of obese adipose tissue begins with functional impairments in ASCs which are governed by dysregulation of microRNA-146b (miR-146b) and its target, Fbxl10. We first identified a distinct cell population in adipose tissue enriched for ASCs with multilineage differentiation and mesenchymal sphere initiation ability. Despite the general hypercellularity and adipocyte hypertrophy seen in visceral adipose tissue, we observed a reduction in ASC proportion and absolute cell number within obese mice. Freshly isolated obese ASCs demonstrated markedly impaired adipogenic and osteogenic differentiation potential confirmed by histological and gene expression analysis. Furthermore, profiling of microRNAs (miRNAs) revealed a set of dysreguated microRNAs crucial for adipogenesis. Among them miR-146b displayed a significant reduction in obese ASCs compared to lean ASCs, a known miRNA dysregulated in human obese patients. Using freshly isolated ASCs, we found that miR-146b is dramatically induced during normal adipogenesis, but was significantly suppressed in obese ASCs and their progenies. Screening of several predicted targets of miR-146b coupled with miRNA-target validation assays suggests that Fbxl10/Kdm2b, a newly discovered suppressor of adipogenesis, is a bona fide miR-146b target in primary ASCs. In summary, our results provide novel evidence that obesity related suppression of ASC frequency and impaired adipogenic capacity may significantly contribute to the dysregulation of adipocyte-turn over, leading to the dysfunction of obese adipose tissue; and pathological disruption of the miR-146/Fbxl10 axis potentially underlies the impaired stem cell capabilities of obese ASCs.

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Diagnostic Journeys of Patients Evaluated for Lyme Disease and Given Extended Antibiotic Therapy

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Background: Lyme disease (LD) is the most common tick-borne disease in the United States and the diagnosis is often far from straightforward, particularly if the initial diagnosis isn't made because LD erythema migrans doesn't develop or isn't noticed. About 10%-20% of patients with LD report nonspecific persistent symptoms even after standard treatment, especially when the diagnosis is delayed, and are sometimes treated with extended courses of antibiotic, though against the recommendations of the Infectious Disease Society of America. We sought to map the diagnostic journey towards the diagnosis of LD, hypothesizing that the patients who ultimately receive extended antibiotics may be clinically distinct. Methods We retrospectively analyzed claims from a nationwide US health insurance plan in 14 high-prevalence states from 2010-2012. **Results:** The incidence rate of patients evaluated for LD and given standard antibiotic therapy (PLDSA) was 40.16 (N=3,184) and for patients evaluated for LD and given extended antibiotic therapy (PLDEA), was and 7.42 (N=588) per 100,000 cases. Within 1 to 30 days before their first LD diagnosis, PLDEA had a higher risk of other nervous system diseases (OR, 1.8; 95% CI, 1.3-2.5; $P < .001$), back problems (OR, 1.7; 95% CI, 1.3-2.3; $P < .001$), and malaise and fatigue (OR, 1.7; 95% CI, 1.3-2.1; $P < .001$) On the other hand, they were less likely to be diagnosed with skin and subcutaneous tissue

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diseases (OR, 0.6; 95% CI, 0.5-0.8; P < .001). In the 6 months before the first LD, PLDEA had higher risks of back problems (OR, 2.0; 95% CI, 1.4-2.8; P < .001) and other connective tissue disease (OR, 1.6; 95% CI, 1.1-2.3; P < .01). **Conclusions:** Patients evaluated for LD who eventually receive extended antibiotic therapy appear to be a distinct subgroup that could be identified earlier in their diagnostic journeys.

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Nociceptive stimulation engages pathologic purinergic signaling to induce inflammation, hemorrhage, and pyroptosis after spinal cord injury

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Traumatic incidents account for the vast majority of spinal cord injuries (SCI) and often produce associated injuries that act as a source of continued nociceptive input. Studies using an upper thoracic transection model of spinal learning have shown that nociceptive input undermines spinal plasticity. Following a lower thoracic contusion, nociceptive input undermines cell survival, impairs recovery of locomotion, and promotes neuropathic pain. However, the mechanism of impaired recovery following nociceptive input is not known. In this study, we used a moderate contusion injury and two different models of nociceptive input to examine the mechanism of impaired recovery following injury. Using the BBB recovery scale, we found that treatment with either capsaicin injection or uncontrollable tail shock undermined locomotor recovery. To better understand the underlying mechanisms, we collected spinal tissue from the lesion site 1, 3, and 24 hours after nociceptive stimulation. Western blotting revealed that nociceptive input increased protein expression of active caspase 1 and increased processing of the pro-inflammatory cytokines IL-1β and IL-18. Interestingly, nociceptive input also caused a robust change in color of the protein extracts. Spectral analysis of protein isolates showed absorbance peaks at 420nm in subjects that received noxious input, indicating an increased concentration of hemoglobin. Western blotting and histology confirmed that there was infiltration of red blood cells. In an attempt to prevent the detrimental effects of nociceptive stimulation, we treated subjects with systemic morphine prior to nociceptive stimulation. Surprisingly, pre-treatment with morphine had no effect on cytokine processing, caspase 1 activation, or hemoglobin infiltration. Finally, we targeted a known upstream activating pathway of caspase 1 – pathologic purinergic signaling – using systemic treatments with probenecid or Brilliant Blue G (BBG). Early after nociceptive input, pharmacologic inhibition of P2X7R or pannexin 1 with BBG or probenecid, respectively, showed a reduction in hemoglobin entry and cytokine processing. Additionally, in a long-term recovery study, all subjects treated with BBG showed reduced at-level pain. However, inhibition of pathologic purinergic signaling only improved locomotor recovery in subjects not receiving nociceptive stimulation. These data suggest that after spinal cord injury, nociceptive stimulation leads to caspase 1 activation, inflammatory cytokine processing, and increased hemorrhage. We propose that one mechanism of caspase 1 activation involves pathologic purinergic signaling. Nociceptive input from associated injuries must be considered as a potential impediment to optimal recovery following SCI. Future work is examining other pathways involved in hemorrhage, inflammation, and cell death caused by nociceptive input after SCI. Supported by the Neilsen Foundation.

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SH2 domain-containing phosphatase-SHP-2 is a novel regulator of fibroblast homeostasis in Pulmonary Fibrosis

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Background: The recent evidence that tyrosine-kinase inhibition may slow down disease progression in patients with idiopathic pulmonary fibrosis (IPF) reignites the interest in regulating signal transduction pathways in lung fibrosis. **Objective:** To determine the role of SHP2 in lung fibrosis **Methods and Results:** Reanalyzing a large microarray dataset obtained from lungs of patients with IPF (n=123) and controls (n=96) we discovered that the tyrosine-protein phosphatase non-receptor type 11 (PTPN11), the gene encoding for the SHP2 was downregulated in IPF lungs. qRT-PCR and western blot analyses further confirmed the microarray results on an mRNA and protein level (3- fold decrease). Immunolocalization studies revealed that SHP2 was mostly absent from active fibroblastic foci and co-localized with SP-B positive cells in the normal alveolar epithelium. SHP2 inhibition through siRNA or PHPS1, a pharmacologic inhibitor, promoted fibroblast to myofibroblast differentiation in normal human lung fibroblasts (NHLF) as assessed by increased formation of stress fibers and increase of both mRNA and protein expression of a-SMA (4- and 3-fold)and col1a1 (5- and 1.5-fold)SHP2 overexpression reduced the responsiveness of fibroblasts to pro-fibrotic stimuli (10ng/ml TGF-b1) leading to significant reductions in 1) cell survival, as assessed by increased expression of apoptotic markers (activated caspase-3 and TUNEL,, 2) proliferation (3-fold, 3) extracellular matrix secretion (1.5-fold increase in mRNA and protein expression of col1a1, and 4) myofibroblast differentiation, (2-fold decrease in a-SMA mRNA and protein expression. We identified that SHP2 anti-fibrotic effects were mediated through dephosphorylation of tyrosine-kinase (c-abl) and serine/threonine-kinase (smad3) signaling pathways in NHLF. Nintedanib, a tyrosine-kinase inhibitor, induced SHP2 expression in NHLF. SHP2 inhibition through intraperitoneal administration of PHPS1 enhanced fibrotic response to bleomycin. Mice carrying the Noonan syndrome-associated gain-of-function SHP2 mutation (SHP2D61G/+) were resistant to bleomycin-induced pulmonary fibrosis and restoration of SHP2 levels in-vivo through lentiviral delivery attenuated bleomycin-induced pulmonary

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fibrosis. **Conclusion:** Our data suggest a novel role for SHP2 as a master regulator of fibroblast homeostasis that prevents fibroblast to myofibroblast trans-differentiation. Augmentation of SHP2 downstream signaling should be investigated as a novel therapeutic strategy for IPF.

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Association between gallstone disease and nonalcoholic fatty liver disease: A systematic review and meta-analysis

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Introduction: Gallstone disease (GSD) is common digestive disorders worldwide, which is the one of the leading cause of hospital admission with high health-care cost burden. The exact pathogenic mechanisms have yet been fully understood. Further exploration of risk factors is crucial leading to early identification of GSD. Nonalcoholic fatty liver disease (NAFLD) shares some similar risk factors with GSD including obesity, insulin resistance and diabetes. Similarly, GSD could also be the risk factor for NAFLD. Several studies on the relationship between these two conditions reported inconsistent results. Thus, we conducted a systematic review and meta-analysis to determine the association between GSD and NAFLD. **Methods:** A comprehensive search of the databases of MEDLINE and EMBASE was performed from inception through November 2015. The inclusion criterion was the observational studies' assessment of the risk of GSD in adults with NAFLD. Outcome was the pooled adjusted odds ratio (OR) of NAFLD between patients with GSD compared with non-GSD controls. GSD was defined as the sonographic evidence of gallstones or history of cholecystectomy. Pooled OR and 95% confidence interval (CI) were calculated using a random-effect. The between-study heterogeneity of effect-size was quantified using the Q statistic and I2. **Results:** The initial search yielded 125 articles. Twelve articles underwent full-length review and data was extracted from eight observational studies (six cross-sectional studies and two cohort studies). The pooled OR of NAFLD in patients who had GSD was 1.58 (95%CI 1.27-1.95). The statistical between-study heterogeneity (I2) was 74.6%, P=0.03. In subgroup analysis, we included only studies defined GSD as sonographic evidence of gallstones, which consisted of four studies, the pooled OR of NAFLD was 1.39 (95%CI 1.05-1.84, I2=86%, Pheterogeneity=0.01) **Conclusion:** Our meta-analysis demonstrated that GSD is significantly associated with NAFLD even after limited to only subgroup of sonographic evidence of gallstones. The mechanism underlies this relationship might be explained by the effect of insulin resistance. Since it can cause both conditions resulting in the accumulation of hepatic triglyceride and also increase secretion of biliary cholesterol. Further prospective studies exploring the underlying mechanism of this association should be pursued.

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The role of MLL family genes in hematopoiesis and infant leukemia

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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 identified by 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, the most frequent chromosomal rearrangement 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. 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 cells from healthy controls. In this system, we modulate the expression of MLL3 using either a CRISPR-Cas knockout approach or an shRNA knockdown strategy. We can also transduce cells at various stages with the MLL-AF9 translocation to drive overt leukemia. 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 leukemogenesis.

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Mutations in NRIP1 cause urinary tract malformations in humans via dysregulation of retinoic acid signaling

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Background: Congenital anomalies of the kidneys and urinary tract (CAKUT) are the most common cause of chronic kidney disease in the first three decades of life. Identification of monogenic mutations that cause CAKUT permits the first insights into related disease mechanisms. However, for most cases of CAKUT the causative mutation remains elusive. Methods: To identify a causative mutated gene for CAKUT we investigated a three-generation Caucasian family with an autosomal dominant form of CAKUT. DNA samples from 6 affected individuals were subjected to whole exome sequencing (WES). Results: We identified a heterozygous truncating mutation (c.279delG, p.Trp93fsX) of the NRIP1 gene in all six affected members of the large family. NRIP1 (Nuclear Receptor Interacting Protein 1) encodes a nuclear receptor transcriptional co-factor, which directly interacts with the retinoic acid receptors to modulate retinoic acid transcriptional activity. Functional studies in HEK293 cells revealed that the mutation abrogated translocation of NRIP1 into the nucleus and abolished retinoic acid dependent transcriptional activity when compared to wild-type NRIP1. *In vivo* studies in Xenopus laevis revealed that NRIP1 has a specific expression pattern in the pronephros and somites starting at very early embryonic stage. Conclusions: We identified a novel monogenic cause of human CAKUT. Our data suggest that dominant NRIP1 mutations cause CAKUT by interference with retinoic acid transcriptional signaling, thus, shedding light on the well documented association between retinoic acid and renal malformations.

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Use of the NEXUS chest clinical decision instrument can reduce unnecessary chest imaging in the blunt trauma victim

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Patients who sustain blunt trauma are often evaluated using chest radiography (CXR) and chest computed tomography (CT), but unnecessary imaging studies increase exposure to ionizing radiation, medical costs, and emergency department length of stay. In order to minimize the number of unnecessary studies performed while maintaining adequate sensitivity for clinically important injuries, the NEXUS group has derived and validated several clinical decision instruments (DI) for selective use of CXR and CT in blunt trauma. In its derivation and internal validation, the NEXUS Chest DI demonstrated a sensitivity and negative predictive value (NPV) for thoracic injury seen on chest imaging (TICI) and major thoracic injury of over 98% and 99%, respectively. In their CT-Major and CT-All DIs, sensitivity and NPV were also consistently over 99%. Despite these statistics, these DIs have not been widely adopted due to their recent development and lack of external validation. In this study, the potential for these DIs to reduce unnecessary imaging studies was assessed in the setting of an urban academic emergency department. 2700 patients who presented after blunt trauma were retrospectively reviewed using a systematic data abstraction tool. The NEXUS Chest imaging DIs (NEXUS Chest, CT-All and CT-Major) were each applied and compared to clinical outcomes. Results suggest that usage of the NEXUS Chest DI in these patients would have prevented 117 unnecessary CXRs and use of CT-All and CT-Major would have prevented 3 unnecessary CT scans. Application of these tools would have led to zero missed clinically significant TICIs and importantly, the results of these imaging studies would not have changed the plans for operative intervention, anesthetic procedures, or admission for observation. This accounts for a total preventable radiation exposure of over 23 mSV or the equivalent of approximately 8 years of background radiation. Furthermore, these DIs could have prevented between $17,500 - $35,000 in patient charges. While external validation of these DIs needs to be confirmed, our study demonstrates that application of these DIs can reduce the overuse of ionizing radiation and limit inflated medical costs without missing clinically significant conditions.

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Identification of Small Molecule Reactivators of Oncogenic p53 Mutants through DNA-binding Domain Stabilization

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The tumor suppressor p53 is an important cell cycle regulating transcription factor and is the most mutated gene in human cancers. Most of these p53 cancer mutations are single amino acid substitutions in the DNA-binding domain (DBD), which cause a destabilization of the p53DBD. The most common of these p53DBD missense mutations is the p53R175H mutation, which destabilizes the melting temperature of p53DBD from 39 °C to 29 °C. A pharmaceutical that can restore wild-type (WT) p53 function to p53R175H or any of these single amino acid mutations could have an enormous impact on our ability to treat many cancers. We designed a p53R175HDBD protein-based chemical library screen to test 2,679 small molecules from the National Cancer Institute Developmental Therapeutics Program for their ability to restore the melting temperature of the p53R175HDBD. We identified the thiosemicarbazone, NSC635448, which is able to stabilize the melting temperature of p53R175HDBD from 29 °C to above 39 °C. We evaluated NSC635448 for its toxicity against engineered osteosarcoma

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cell lines expressing p53R175H, p53G245S, and no p53 (p53null). NSC635448 is most effective at inducing cell death in osteosarcoma cells harboring p53R175H or p53G245S (EC50: 32.1 nM and 407 nM, respectively) while demonstrating lower toxicity to p53null osteosarcoma cells (EC50: 2.46 μM). NSC635448's enhanced antiproliferative effect on mutant p53-containing osteosarcoma cells illustrates its mutant p53-dependent activity. We are currently working to understand NSC635448's binding site on p53, its mechanism of p53 stabilization, its effects on p53 cellular functions, and the therapeutic index of this thiosemicarbazone on the most common p53 mutations in human cancers. Understanding how NSC635448 restores mutant p53 function will allow us to develop thiosemicarbazones with improved specificity and determine which cancers are amenable to treatment by thiosemicarbazones.

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Ugandan children's strategic use of food at a globally funded HIV rehabilitation center

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Children living with HIV, like all children, find creative ways to exercise their will, even in difficult circumstances. My research focuses on Ugandan children infected with HIV who were delayed in accessing medical treatment until they became seriously ill. These children, often from rural villages, were admitted to a peri-urban rehabilitation center for 3-18 months, before being reintegrated to their homes. Fieldwork revealed that food was an important metric by which children's health and rehabilitation status was measured by staff at the center and by caregivers. Somewhat ironically, internationally developed and acclaimed nutritional supplements tended to be rejected by those children who were prescribed to eat them, yet requested by those deemed "healthy" enough to not need them. Food also became a tool which children creatively adopted and manipulated to express their preference for place, and to attempt to exercise control over their environment. Children expressed their desire to return home by contrasting the foods they ate the center with those they ate at home. As a tool for exercising their agency, children used food, especially the refusal to eat, as an attempt to gain the attention of loved ones and/or to be returned to their homes. In a variety of ways, food became an important factor as children navigated the process of rehabilitation: from suffering from an acute illness in a peri-urban institution to living with a chronic condition in a rural home.

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Loss-of-function variants in NPC1 and risk for obesity

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Much of the genetic architecture for the "missing heritability" of human obesity, particularly low-frequency variants with intermediate effects, remains to be determined. We conducted a comprehensive and in-depth assessment of low-frequency variants in the Niemann-Pick Type C1 (NPC1) gene in young, severely obese cases and controls. We identified 14 novel non-synonymous variants in NPC1 with frequency less than 1% that were significantly associated with increased risk of obesity (P = 1.8×10-8, Odds Ratio = 10.7). Participants carrying variants with severely damaged cholesterol-transport ability had more fat accumulation. We further recruited parents of 25 probands with rare autosomal recessive NP-C disease and found that NPC1+/- carriers had a higher BMI than matched controls in men (median BMI 26.57 vs. 23.99 kg/m2, P = 0.007). Consistently, male Npc1+/- mice had increased fat storage under high-fat, high calories diet. In summary, novel loss-of-function NPC1 mutations and associated human obesity phenotypes were identified to be significantly associated in a sex-specific manner indicating a strong sex-specific gene-diet interaction for the development of obesity.

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Role of Protein Kinase C Delta in the Ionizing Radiation DNA Damage Response

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Cells exposed to ionizing radiation undergo single and double strand DNA breaks via direct and indirect ionizing events. Irradiated cells can then either trigger a cell cycle arrest and activate DNA repair pathways, or they can undergo apoptosis if the damage is too great. Understanding the regulation of IR DNA damage responses is important for cancer radiotherapy as well as radiation carcinogenesis. The serine/threonine kinase protein kinase C delta has been implicated in the induction of apoptosis in response to ultraviolet and ionizing radiation, and PKCδ expression is lost in several cancers. In this study we explored the role of PKCδ in the IR DNA damage response. Wild type and PKCδ null mouse embryo fibroblasts were exposed to a low, non-lethal dose (5 Gy) of IR. Cells were then cultured for 1 hour and 24 hours, and DNA double strand breaks were assessed using the single cell electrophoresis, or Comet, assay. We observed that after 1 hour, both wild type and PKCδ null cells showed increased DNA damage. After 24 hours, wild type cells showed a significant ($p<10^{-30}$) decrease in DNA damage which was almost back to control levels, while PKCδ null cells

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showed a sustained high level of DNA damage. PKCδ null cells were then retrovirally-transduced with PKCδ, and their ability to repair IR-induced DNA double strand breaks was determined. When PKCδ was reintroduced into PKCδ null cells, DNA double strand breaks were still induced 1 hour after IR exposure, but double strand breaks were significantly reduced after 24 hours ($p<10-5$). Thus, re-introduction of PKCδ into PKCδ null cells rescued their defect in DNA double strand break repair. Cells with defected DNA damage repair are often more sensitive to DNA damage. To test if PKCδ null cells were more sensitive to IR DNA damage, a survival assay was performed by exposing wild type and PKCδ null MEFs to increasing levels of IR radiation (0, 2, 5, 10 Gy). The survival assay indicated that PKCδ null cells were less sensitive to IR radiation compared with wild type cells. Thus, despite a defect in repairing IR-induced DNA damage, PKCδ null cells were resistant to killing by IR. This may be due to a dominant role for PKCδ in inducing apoptosis in response to IR. These results suggest that PKCδ plays a major role in the DNA repair pathway following IR exposure, and that the loss of PKCδ may contribute to carcinogenesis by allowing damaged cells to survive despite unrepaired DNA damage.

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The impact of medical school research experiences on long-term career outcomes

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Many US medical schools are redesigning their curricula to place a greater emphasis on research as a potential intervention to address the shrinking number of medical students choosing to become academic physicians and physician scientists. While institutions often report immediate outcomes related to participation in medical school (MS) research, there is a notable paucity of data regarding long term impact. We designed a retrospective qualitative survey study to evaluate the effects of MS research on career outcomes. The survey tool consisted of 74 possible questions incorporating skip patterns administered online using the web-based survey program Qualtrics to UASOM alumni graduating from 2000-2014 (obtained from the UASOM Development and Community Relations Office email database). Alumni were contacted three times at two week intervals. We received 168 completed surveys for an 11.5% response rate. MD/PhD respondents and inconsistent responses were excluded from the current study. Of 129 total respondents, 92 (71%) completed research during medical school. Completion of MS research increased desire for future research involvement in 24% of respondents; 53% reported no change. Of the MS research characteristics we evaluated, respondents involved in research in their current career were significantly more likely to have completed the required Scholarly Activity clerkship (OR=2.108, p=0.0471), engaged in clinical research (OR=3.088, p=0.0092), been motivated by a desire "to pursue a passion for research" (OR=5.707, p=0.0009) or "to learn more about a medical specialty" (OR=2.941, p=0.0072), and reported an "increase in desire for future research involvement as a result of MS research" (p=0.0007) when compared to respondents currently not involved in research. Although completion of MS research was the strongest predictor for employment in an academic medical center (Z=3.060, p=0.0022), the strongest predictor for current research involvement was "change in desire for future research involvement as a result of MS research" (Z=2.833, p=0.0046). Linear regression analysis on the 5-point answer scale for the latter revealed that motivation, mentorship, and publication all contributed significantly to this metric (p=2.54e-7, p=0.0036, p=0.0304, respectively). Our results indicate that completion of MS research is a significant predictor for employment in academics and current research involvement in UASOM alumni. One component of this relationship, inherent passion for research, seems to predate medical school matriculation, suggesting medical students likely to pursue research careers can be identified and supported early in their training. However, characteristics of MS research, including mentorship and publication, also influence future research involvement, and optimization in these areas may help guide medical students into careers as physician scientists.

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Characterization of the Role of MED19 in Prostate Cancer

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Prostate cancer is the most common cancer in men in the United States and is a leading cause of cancer-related mortality. Prostate cancer is treated with agents that target androgen synthesis, since prostate cells rely on androgens for survival. However, it has been found that in castration-resistant prostate cancer (CRPC), the androgen receptor (AR) drives tumor progression independent of androgen levels, inducing treatment resistance. This indicates that AR induces cell survival and growth by alternative mechanisms, and current research focuses on elucidating these. One such mechanism is the up-regulation of activating AR co-regulators. Through a genome-wide RNAi screen, our lab identified MED19, a component of the mediator complex, as one of these candidate AR co-activators. Up-regulation of MED19 mRNA in a subset of prostate cancer patients is correlated with decreased survival, and our lab has shown that transient MED19 depletion affects AR transcriptional activity and reduces the proliferation of LNCaP-abl cells, a CRPC cell line. Our current data indicates that stable over-expression of MED19 in androgen-dependent LNCaP cells induces a specific reduction of KLK3 expression and induces proliferation under conditions of androgen deprivation. This indicates that MED19 may play a role in differentially regulating AR transcriptional activity in prostate cancer and could potentiate androgen-resistant growth, thus contributing to castration resistance. We are currently focusing on elucidating the mechanisms by which MED19 regulates AR activity and drives androgen-resistant growth, as well determining its potential function in drug resistance, colony formation, and invasion. Future work will focus on inhibition of MED19, including the testing of novel targeting

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strategies. Characterization of the role of MED19 role in prostate cancer could provide insight into the mechanisms behind CRPC progression and aid in the development of more effective therapies.

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Turning the "Luke Skywalker" hand into a reality: Bidirectional, intuitive, and dexterous control of a virtual prosthetic hand by transradial amputees with implanted neural and muscular electrodes

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Introduction: Currently, commercially available prosthetic arms have many limitations, including the small number of available basic hand movements, and lack of sensory feedback. We are addressing these limitations by developing a bi-directional neuromuscular interface for a prosthetic hand. Here we present results from an ongoing study, in which our interface system is capable of restoring independent control of each digit and the wrist of a virtual prosthetic hand (VPH), and that can elicit up to 131 distinct, naturalistic sensations in the amputee's phantom hand via electrical microstimulation of the residual arm nerves. Additionally, we combined real-time decoding algorithms and microstimulation, cued by sensors on the VPH, to provide bidirectional control that does not require visual feedback and feels natural and meaningful to the amputee. **Methods and results:** In this IRB-approved study, one or two total Utah slanted electrode arrays (USEAs), each containing 100 microelectrodes, were implanted in the residual ulnar and/or median arm nerves of five human, transradial amputees for up to 12 weeks. In addition, 8 flexible intramuscular electrode (iEMGs) leads with 4 channels each, were also implanted into the residual forearm muscles (5 leads in the flexor side, and 3 leads in the extensor side) of the most recent subject. Three types of experiments were performed: 1) decoding intended movements in real-time to allow subject to move the virtual hand, 2) eliciting percepts on the phantom hand via microstimulation, and 3) closed-loop control of the virtual hand. 1) Decode experiments involved a calibration phase, followed by a testing phase, in which the subject attempted to control the VPH to touch or follow targets in virtual space. Using combined neuromuscular data, Subject 5 successfully completed an 8 DoF target touching task (29/30 successful trials). 2) Microstimulation experiments involved mapping the percepts for each electrode followed by testing for repeatability and distinguishability. Subject 4 reported 131 localized proprioceptive and cutaneous percepts spanning the fingers, palm, and posterior of the hand. 3) In closed-loop experiments, subjects were asked to distinguish between large and small objects in the virtual environment without visual feedback. Subject 3 was able to correctly distinguish between a large and small virtual object in 41/47 trials ($p < 0.001$, binomial test). Microstimulation-evoked percepts were generally described positively by the subjects. Subject 5 stated, "God, that's cool," while touching an object in the virtual environment. **Conclusion:** Results from this study support the use of USEAs and iEMGs as feasible, implantable interfaces that can partially restore sensorimotor function for amputees.

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Isolation of activated CD8+ T cells following drug stimulation: a first step toward evaluating the heterologous immunity model for pure T-cell mediated adverse drug reactions

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Severe immune-mediated adverse drug reactions (IM-ADRs) result in patient morbidity and mortality and inflict significant cost on healthcare and drug development. Pure T-cell mediated IM-ADRs are strongly associated with genetic variation in class I HLA loci which has led to the development of successful preventive genetic screening in the case of abacavir hypersensitivity (HLA-B*57:01) and carbamazepine associated Stevens-Johnson Syndrome/Toxic epidermal necrolysis (SJS/TEN) (HLA-B*15:02). The feasibility of preventive HLA screening for most drugs is hampered by the fact that only a small percentage of those carrying an HLA class I risk allele will develop an IM-ADR. Current models that describe the interactions of small molecule pharmaceuticals with HLA also do not explain their rapid onset, tissue specificity and long-lasting immunity. We propose a unifying heterologous immunity model for pure T-cell mediated IM-ADRs and will develop an experimental roadmap to define 1) why carriage of the HLA risk allele is necessary but not sufficient for the generation of the IM-ADR, 2) how exposure to a prevalent viral pathogen, such as a human herpes virus (HHV), establishes a population of HLA risk allele-restricted memory CD8+ T cells that includes inherently cross-reactive clonotypes, and 3) that later exposure to the causative drug induces the formation of an HLA-drug-self peptide complex that is recognized by a cross-reactive, HHV-specific T-cell clonotype to elicit a rapid memory CD8+ T-cell response. We have isolated activated (CD137+/CD69+) CD8+ T cells from severe drug-induced IM-ADRs carrying an HLA-class I risk allele and following ex-vivo stimulation with the implicated drug. Single cells were sorted following by fluorescent antibody labelling for surface activation markers, sequenced for paired T-cell receptor alpha and beta genes and phenotypic markers, and tested for cross-reactivity with candidate HLA-class I restricted HHV peptides to evaluate the heterologous immunity model.

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Streptococcus pyogenes quorum sensing results in increased immunomodulation

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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, termed the Rgg2/3 pathway. GAS QS utilizes short hydrophobic peptides (SHPs) that are produced by the bacteria, secreted across the membrane, and 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. This system is induced by environmental conditions akin to those GAS would encounter in the nasopharynx, and can be abrogated in response to stressful conditions such as antimicrobial peptide exposure. Despite the advances we have made in our understanding of this novel QS system, we do not yet have a clear understanding of how QS in GAS impacts human health. However, we have shown that Rgg 2/3 circuit activation leads to biofilm formation, cellular aggregation and improved resistance to lysozyme. These changes appear to be mediated by the short gene stcB, transcribed as part of the shp2 operon (under QS control), which we hypothesize leads to changes in the cell wall via interaction with the CHAP-domain containing proteases Isp1 and Isp2. Preliminary data demonstrate that overexpression of stcB leads to improved adherence in a tissue culture model as well as improve ability to limit inflammation via enhanced degradation of IL-8. Collectively, these findings have led us to hypothesize that the Rgg2/3 system plays an important role in mediating host-bacteria interactions, via the modification of the cell wall, by altering tropism for host tissue and limiting immune response. These changes prime GAS to live asymptomatically in the host. This work seeks to quantify the changes to the cell wall leading to these observed phenotypes, while expanding our understanding of the immunomodulatory potential of QS in GAS.

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Metabolic Reprogramming in Endothelial Cells Following Loss of Laminar Shear Stress

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Endothelial cell (EC) dysfunction is a hallmark of conditions characterized by disturbed blood flow such as atherosclerosis, and sepsis. In their natural environment, ECs are constantly exposed to laminar sheer stress due to blood flow. Alterations in blood flow leads to loss of laminar sheer stress and consequently EC dysfunction, increased reactive oxygen species (ROS), vascular leak, inflammation, and angiogenesis. Increasing evidence suggests that metabolic reprogramming may play a role in EC dysfunction induced by the loss of sheer stress. However, the mechanisms by which laminar flow regulates metabolic changes in ECs are not completely understood. We hypothesized that loss of laminar sheer stress upregulates glycolysis by increased generation of ROS from the mitochondria. **Methods:** We subjected human aortic ECs to either no flow or laminar flow via a cone-plate viscometer system and measured gene (qRT-PCR) and protein (western blotting) expression of glycolytic enzymes, mitochondrial complexes, and pro-inflammatory cytokines. Glycolytic and mitochondrial functions were assessed by bioenergetics measurements (Seahorse), and microscopy. Mitochondrial ROS generation was measured using MitoSOX. **Results:** Compared to ECs subjected to laminar flow, cells exposed to no flow had increased expression of glycolytic enzymes and a higher rate of glycolysis. Loss of laminar flow altered cellular localization of mitochondria, increased the generation of mitochondrial ROS and stabilized hypoxia inducible factor-1α (HIF-1α). Inhibition of ROS or HIF-1α stabilization attenuated the increase in glycolysis and glycolytic capacity in ECs subjected to no flow. **Conclusions:** Loss of laminar sheer stress upregulates glycolysis via mitochondrial ROS generation and HIF stabilization. In contrast, laminar flow maintains a normal mitochondrial function. Targeting metabolic pathways may have a therapeutic role in the treatment of EC dysfunction that occur during disturbed flow conditions.

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Two types of cortical interneuron complementarily mediate behavioral detection of deviant sounds

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Neurons in the primary auditory cortex respond strongly to rare sounds and weakly to common sounds, a phenomenon known as stimulus-specific adaptation (SSA). However, the neuronal mechanisms underlying the relationship between SSA and behavioral detection of deviant sounds remain elusive. We recently found that two populations of cortical interneuron, parvalbumin-positive (PV) or somatostatin-positive (SOM),

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mediate SSA in the auditory cortex (Natan et al., eLife 2015). Here, we tested whether and how these interneurons contribute to behavioral detection of deviant sounds by combining behavioral assays and optogenetic manipulation. We assessed the ability of mice to detect standard or deviant tones by measuring the amount to which a standard or a deviant pre-pulse tone inhibits the acoustic startle reflex to a loud pulse, i.e. pre-pulse inhibition (PPI). We hypothesized that, following a train of standard tones, a deviant tone would lead to stronger PPI than another standard tone. We optogenetically suppressed PVs or SOMs to test their involvement in the detection of standard and deviant tones. Suppression of either class of interneurons led to a significantly reduced difference in PPI between standard and deviant tones, suggesting that these interneurons not only mediate SSA but also contribute to the behavioral detection of deviant sounds. Importantly, PVs and SOMs differentially contributed to reduction in PPI difference between standard and deviant tones in a stimulus-specific and complementary fashion. Suppression of PVs reduced the difference by significantly increasing PPI in response to standard, but not deviant tones. In contrast, suppression of SOMs reduced the difference by significantly reducing PPI in response to deviant, but not standard tones. Taken together, our results establish a neuronal basis to the heretofore hypothesized relationship between SSA and behavioral detection, by demonstrating that two distinct populations of interneurons differentially mediate behavioral detection of rare vs. frequent tones.

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Metabolic reprogramming regulates brain tumor initiating cells

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Brain tumor initiating cells (BTICs) hijack the process of high-affinity glucose uptake from normal nerve cells to meet energy demand and sustain metabolic stress in a dynamic tumor microenvironment. How BTIC reprograms its downstream metabolic network to manage such increased carbon flow and to maintain self-renewal and tumorigenic capacity remains elusive. Using an unbiased metabolomic and genomic discovery approach, we found that de novo serine synthesis pathway is functionally upregulated in BTIC as compared to non-BTIC tumor cells, to preferentially channel glucose-derived carbon into anabolic metabolism. Elevated expression of key enzymes in this pathway predict poor prognosis in glioblastoma patients, revealing a potential point of fragility for therapeutic intervention. Targeting serine synthesis through RNA interference inhibits BTIC growth, self-renewal, and *in vivo* tumor growth through depleting intracellular pool of serine and carbon supply into one-carbon metabolism. Upstream, transcriptional activation of serine synthesis pathway is tightly regulated to the glucose influx mediated by GLUT3. These results provide critical mechanistic insight into how altered metabolic status, a hallmark of cancer, fuels tumor hierarchy and causes unfavorable clinical outcome in patients.

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Identification of Active Surveillance of Prostate Cancer Candidates Using mpMRI

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Introduction and Objectives: Convincing data suggest side effects and complications of overtreatment of prostate cancer. Active surveillance (AS) has been suggested as a viable option to manage indolent prostate tumor. Patients on AS are carefully monitored with routine screening and annual biopsy to assess cancer progression. Eligibility criteria for AS include low gleason score (<6), low PSA (<10), and fewer than 3/12 positive biopsies. Multiparametric MRI and ultrasound (mpMRI/US) fusion biopsy can detect distinct lesions within prostate, and can be effective as a screening tool to ensure patients are true AS candidates. **Methods:** A retrospective review was performed on consecutive patients with history of mpMRI imaging at Loyola from 2013 to 2014. All patients who were currently on AS protocols were included. Patients at some point during AS underwent MRI/TRUS fusion biopsy as part of their AS protocol. Demographic and pathologic characteristics of these patients were examined. **Results:** We identified 30 patients on AS with initial registration by traditional TRUS prostate biopsy who subsequently underwent MRI/TRUS fusion biopsy as part of AS protocol. Mean age at MRI was 65 yrs (48-79), mean of the most recent PSA before MRI 6.2 ng/mL, and the mean PSAD 0.139. There were total 20 Caucasian, 9 African American, and 1 Asian. The patients underwent endorectal coil mpMRI and lesions were graded for suspicion of prostate cancer. Patients proceeded to MRI/US fusion biopsy using the Uronav platform if any area of suspicion were identified. 10 patients were upstaged by mpMRI/US Uronav fusion biopsy, and were deemed ineligible for AS. Their mean age was 65.5 years, mean BMI 30.5, mean PSA 7.2 ng/mL, mean PSAD 0.162. One patient was upstaged to gleason score 8, and the rest to gleason score 7. Overall, of the entire 30 patients qualified on AS, 10 (33.3%) were upstaged by MRI/US fusion biopsy at Loyola, and elected active treatment by radiation or surgery **Conclusions:** Multiparametric MRI in conjunction with a MRI/US Uronav fusion biopsy platform is a valuable screening tool for assessing whether men are appropriate candidates for AS. Approximately 1 of 3 patients who were initially placed on AS using standard TRUS biopsy were upstaged, and excluded from AS. MRI/US Uronav fusion biopsy may be an effective way to help define patients who are truly eligible for AS.

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Phyllanthusmins, a potential new class of natural product chemotherapeutic, for use in the treatment of ovarian cancer

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As part of a large multi-institutional, multidisiplinary project, a new natural product has been identified with chemotherapeutic activity. The phyllanthusmin compounds were isolated from the Phyllanthus species of flowering plants. These compounds demonstrated cytotoxic activity and were subsequently shown to display *in vivo* anti-cancer action in a hollow fiber assay in colon cancer cells. One limitation of natural products research is securing enough material for additional mechanistic studies. In order to accomplish this goal, synthesis of the compound was achieved, granting access to large quantities, as well as synthetic analogs, for biological study. Preliminary data indicate that phyllanthusmins, though structurally similar to other chemotherapeutics, specifically topoisomerase inhibitors, seem to have a novel mechanism of action, slowing the growth of tumor cells in various types of cancer (colon, breast, melanoma, and ovarian cancer) *in vitro*. Our study focused on the action of these compounds in ovarian cancer. Four ovarian cancer cell lines (OVCAR3, OVCAR4, OVCAR8, and Kuramochi) were tested with 9 phyllanthusmin analogues, as well as etoposide and paclitaxel as positive controls, in a range of doses for 3 days. In general, OVCAR8 was most sensitive to the compounds with an average IC50 of 5.9 μM, while Kuramochi was the most resistant with an average IC50 of 123.3 μM. The most potent analog, PHY25, had an IC50 of 27.9 nM in OVCAR8 and 1.6 μM in Kuramochi. Interestingly, the differing genetic backgrounds of these cell lines, most notably the presence of a BRCA2 mutation and MYC amplification in Kuramochi but not OVCAR8, may explain the differences in drug sensitivity between these cell lines (Domcke et. al. 2013). The same observation of greatest drug sensitivity in OVCAR8 and greatest resistance in Kuramochi was also seen in both positive controls. Preliminary *in vivo* experiments, in which OVCAR3 cells were grafted intraperitoneally and mice were treated after 5 weeks with a regimen of either PHY1 or PHY13 intraperitoneal injections, revealed a decrease in the number of tumors found via gross examination, but no decrease in average or total tumor weight compared to control (vehicle) treatment. In the future, we plan to continue to perform *in vitro* and *in vivo* tests to verify the increasing potency and solubility of new PHY analogues synthesized. We will work to determine the mechanism of action of the most potent analogues with RNAseq and biological fingerprinting assays.

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Regulation of ubiquitination and lysosomal trafficking of the beta-1 adrenergic receptors by the deubiquitinase USP20 in response to cardiac pressure overload

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Background: The deubiquitinating enzymes, USP20 and USP33 coordinate the cellular expression, trafficking and signaling at the β2 adrenergic receptor (β2AR). β2AR-stimulated site-specific phosphorylation of USP20 by PKAα inhibits both β2AR-USP20 interaction and β2AR deubiquitination, thus promoting lysosomal degradation of the β2AR. Whether the de-ubiquitination machinery has an effect on the β1AR, the predominant βAR subtype in the heart, and whether USP20 phosphorylation has a regulatory role on cardiac function are unknown. Methods: β1AR ubiquitination and lysosomal trafficking by unphosphorylated and Serine333-phosphorylated human USP20 or Serine334-phosphorylated murine USP20 (pUSP20) were ascertained by individual siRNA-mediated downregulation of USP20 and PKA isoforms as well as by co-expression of USP20 constructs that mimic phosphorylated and non-phosphorylated states. The levels of total and pUSP20 in the myocardium of WT (C57BL/6), β1AR KO mice were assessed after two-week chronic pressure overload induced by transverse aortic constriction (TAC). Results: β1AR ubiquitination and lysosomal trafficking significantly increased upon USP20 knockdown; thus, USP20 is a cognate deubiquitinase that orchestrates intracellular trafficking of the β1AR. Agonist-stimulation of the β1AR induced rapid site-specific phosphorylation of USP20 by PKAα. PKAα downregulation that blocks USP20 phosphorylation in fact promoted β1AR ubiquitination and lysosomal trafficking. Both phosphorylated and unphosphorylated USP20 bound the β1AR with equal affinity. USP20 S334D prevented whereas USP20 S334A dramatically increased lysosomal trafficking of the β1AR. Chronic pressure overload achieved by TAC augmented pUSP20 in WT but not in β1AR KO mice myocardium suggesting that cardiac pUSP20 is predominantly regulated by the β1AR during chronic TAC. Our findings suggest that pUSP20 acts reciprocally on the βAR subtypes: it facilitates β1AR deubiquitination and expression whereas enhances β2AR ubiquitination and lysosomal degradation. Accordingly, pUSP20 in pressure overloaded hearts might promote β2AR downregulation while preserving β1AR signaling during left ventricular hypertrophy. Author Disclosure Information: S.M. Yu: None. L. Mao: None. H. Rockman: Ownership Interest; Significant; Trevena Inc. S.K. Shenoy: None.

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Practical Strategies for Improving Professional Decision-Making in Medicine

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Professional decision-making is important in medical practice, the implications of which affect physicians, patients, and other health care providers. Situations occurring from poor professional decision-making can negatively affect patient care. Bioethics and medical professional societies aim to guide professional behavior, the precursor to professional decision-making, through guidelines and references to professional codes of ethics. The success of these endeavors is difficult to assess; however, neither bioethics nor professional organizations have provided physicians with practical tools to supplement efforts to improve professional decision-making in the clinical setting. With this gap in the literature, medicine has limited exposure to effective strategies for implementing and improving professional decision-making in the clinical setting. Our research has uncovered some effective strategies to guide such decision-making through an interdisciplinary literature review. The findings show that certain personality traits negatively influence professional decision-making, requirements for learning and improving professional behavior are overlooked in medical training, and certain reflective practices could improve professional decision-making among health care providers. This information can serve physicians and patients by improving professional decision-making and thus improve the quality of care that patients receive.

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Glycerol Monolaurate (GML) inhibits human T cell signaling, metabolism, and function by disrupting lipid dynamics

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Glycerol Monolaurate (GML) is a naturally occurring fatty acid widely utilized in food, cosmetics, and homeopathic supplements. GML is a potent antimicrobial agent that targets a range of bacteria, fungi, and enveloped viruses. Interestingly, GML suppresses mitogen induced lymphocyte proliferation and inositol triphosphate production, suggesting that GML has immunomodulatory functions. In this study, we have mechanistically examined if GML affects the signaling, metabolism, and functional output of human primary T cells. We found that GML potently altered order and disorder dynamics in the plasma membrane that resulted in reduced membrane localized clustering of the proteins LAT, PLC-γ, and AKT, events integral for proper T cell receptor (TCR) signal propagation. Altered membrane signaling events induced selective inhibition of TCR-induced phosphorylation of SLP-76, regulatory P85 subunit of PI3K, and AKT as well as abrogated calcium influx. In addition to signaling defects, GML treated cells have profoundly altered metabolism profiles characterized by suppressed oxidative phosphorylation and increased glycolysis. Functionally, GML treatment potently reduced TCR-induced production of the cytokines IL-2, IFN-γ, TNF-α, and IL-10. Our data reveal that the widely used anti-microbial agent GML alters the lipid dynamics of human T cells, leading to their defective signaling, metabolism, and function.

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Tcf7l1 is Required for Enhancer Inactivation During Differentiation of Embryonic Stem Cells

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Embryonic stem cells (ESC) can both self-renew and differentiate into any tissue comprising an organism, offering tremendous promise for regenerative medicine. Though many *in vitro* differentiation protocols yield functional tissues, the presence of cells retaining tumorigenic, pro-self-renewal properties of ESCs remains a major barrier to clinical use. Here, we focus on conversion of ESCs into epiblast-like stem cells (EpiLC). While both cell types possess potential to generate all adult tissues, their contrasting responses to Wnt/Beta-catenin - self-renewal in ESC versus differentiation in EpiLC - is highly relevant to preventing tumorigenicity in laboratory-derived cell populations. In addition to using gene expression to assess differentiation, techniques identifying open chromatin (ie: ATAC-seq) allow us to view developmental transitions as the opening of lineage-specific and closing of naïve enhancers and promoters. Indeed, cell identity can be defined by which cis-regulatory elements are open for transcription factor binding and gene activation. Here, using microarrays and ATAC-seq, we show that Tcf7l1 DNA-binding protein is indispensible to the ESC-to-EpiLC switch. In ESCs, Wnt/Beta-catenin-mediated degradation of Tcf7l1 drives self-renewal. In congruence, we show Tcf7l1 is necessary and sufficient for gene expression changes during Beta-catenin activation and ESC-to-EpiLC conversion. A transcriptional repressor, Tcf7l1 binding is highest at enhancers regulating genes decreased in the ESC-to-EpiLC transition. Furthermore, Tcf7l1 binding is predictive of and necessary for naïve regulatory region closing. Here in a complementary role to pioneer factors, which activate lineage-specific regulatory regions, Tcf7l1 is proposed to be a founding member of "past-burying" factors necessary to inactivate naïve enhancers during differentiation.

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Small molecule ONC201/TIC10 inhibits HIV-1 replication in macrophages and lymphocytes

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Background: Despite combination antiretroviral therapy (cART), HIV-1 continues to form reservoirs in the lymphoids, gut and central nervous system. Eradication of HIV from these reservoirs remains elusive. FOXO3a, a powerful transcription factor critical for immune homeostasis, offers hope to eliminate HIV-1 reservoirs. Lack of FOXO3a in the nucleus facilitates the maintenance of the latent HIV-1 reservoirs. Our previous publications have demonstrated that FOXO3a targets HIV-1-infected macrophages for apoptosis. Recent drug development has provided TIC10, also known as ONC201, as a potent and stable small molecule FOXO3a activator. TIC10 is orally active, can cross blood-brain barrier and has shown efficacious antitumor effect in clinical trials. This most recent drug development serves as a strong rationale for the current study. We hypothesize that targeting FOXO3a through TIC10 will inhibit HIV-1 in its reservoir cell types. Methods: Human primary microglia, monocyte-derived macrophage, peripheral blood lymphocyte culture systems, macrophage-tropic HIV-1ADA and T-tropic HIV-1IIIB were used to study the antiviral activity of TIC10. Viral infection was monitored by reverse transcriptase activity and HIV-1 p24 levels. Cell viability was measured by MTT. Cell death and its linked intracellular signaling pathways were determined with the phosphorylation of FOXO3a, cleavage of Poly (ADP-ribose) polymerase (PARP) and caspase 3 by Western blot. Data were evaluated statistically by ANOVA, followed by the Tukey-posttest for paired observations. Results: TIC10, but not its isomer, potently inhibited HIV-1 replication in infected macrophages, microglia, and lymphocytes in a dose-dependent manner, suggesting that the antiviral activity is specific to TIC10. Interestingly, unlike tumor cell lines, which were sensitive to TIC10-induced cell death, primary macrophages were resistant to TIC10-induced cell death. The reduced levels of HIV-1 replication in the infected cells were associated with FOXO3a activation and the cleavages of PARP and caspase 3, indicating that TIC10 inhibits HIV-1 replication through modulation of FOXO3a and the related apoptotic signaling. Conclusion: Small molecule ONC201/TIC10 induces cell death and inhibits HIV-1 in infected-macrophages and lymphocytes. Therefore, ONC201/TIC10 can be a promising drug candidate to combat persistent and latent HIV-1 infection in the current cART era.

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The Histone Deacetylase Sirt2 Regulates Slug Stability and is Necessary For BRCA1-Associated Triple Negative Breast Cancer

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Triple-negative breast cancer represents the most aggressive and deadliest breast cancer subtype affecting 20% of breast cancer patients. Unlike other subtypes, few driver mutations have been identified and, thus, no targeted therapies have been established. We previously identified the transcriptional repressor Slug/SNAI2 is frequently overexpressed in diseased breast tissues of women with inherited mutations in BRCA1; this aberrant expression of Slug represents an important determinant in the pathogenesis of aggressive BLBC. Despite the importance of Slug during development and disease, little is known about its homeostatic regulation and how it becomes deregulated in cancer. Here we identify the NAD+ dependent deacetylase Sirt2 as a master regulator of Slug protein levels. Sirt2 inhibition leads to rapid turnover and depletion of Slug protein, whereas Sirt2 overexpression stabilizes Slug and increases its level and activity. Mechanistically, Sirt2 binds to and deacetylates Slug protein at K116 residue to prevent its proteasomal degradation, and thereby stabilizes Slug protein. Analysis from human breast cancer tissues show that Sirt2 is frequently amplified, overexpressed, and positively correlated with Slug status in triple-negative breast tumors. Importantly, Sirt2 is induced following BRCA1 inhibition and inactivation of Sirt2 in BRCA1-associated breast cancer cells leads to rapid Slug turnover and loss of pathological features that are clinically relevant to triple-negative tumors. Collectively, these observations revealed a regulatory mechanism controlling Slug abundance, whereby dysregulated Sirt2 manifests into Slug overexpression in human cancer. As such, targeting Sirt2 may be a rational strategy to molecularly dampen Slug hyperactivity and malignant traits characteristic of triple-negative breast cancers, especially those associated with BRCA1 mutations.

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Coagulation factor XI promotes distal platelet activation and single platelet consumption in the bloodstream under shear flow

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Objective: Coagulation factor XI (FXI) has been shown to contribute to thrombus formation on collagen or tissue factor (TF)-coated surfaces *in vitro* and *in vivo* by enhancing thrombin generation. Whether the role of the intrinsic pathway of coagulation is restricted to the local site of thrombus formation is unknown. This study was aimed to determine whether FXI couldpromote both proximal and distal platelet activation and aggregate formation in the bloodstream. Approach and **Results:** Pharmacological blockade of FXI activation or thrombin activity in blooddid not affect local platelet adhesion, yet reduced local platelet aggregation, thrombin localization and fibrin formation on immobilized collagen and TF under shear flow, ex vivo. Downstream of the thrombus formed on immobilized collagen or collagen and 10 pM TF, platelet CD62P expression and microaggregate formation and progressive platelet consumption weresignificantly reduced in the presence of FXI-function blocking antibodies or a thrombin inhibitorin a shear rate- and time-dependent manner. In a non-human primate model of thrombosis, we found that inhibition of FXI reduced single platelet consumption in the bloodstream distal to a site of thrombus formation. **Conclusions:** This study demonstrates that the FXI-thrombin axis contributes to distal platelet activation and procoagulant microaggregate formation in the blood flow downstream of the site of thrombus formation. Our data highlights FXI as a novel therapeutic target for inhibiting distal thrombus formation without affecting proximal platelet adhesion.

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The Plasticity of the Intestinal Stem Cell Compartment and its Induction Following Rotavirus Infection

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The intestinal epithelium exhibits a remarkable ability to self-renew due to the continuous proliferation and differentiation of intestinal stem cell (ISC) populations. LGR5-expressing cells are thought to actively cycle, whereas HOPX-expressing cells remain quiescent. Intestinal epithelial injury has mainly been studied using radiation models in which the stem cell itself is injured. Little is known about ISC responses that result from damage to differentiated cells. Using human intestinal enteroid (HIE) model in the context of a prevalent human intestinal pathogen, rotavirus (RV), we show that damage to differentiated cell types can promote ISC induction. HIEs were generated from human jejunal tissue and grown into 3D structures with support of growth factor WNT. Differentiation was induced by withdrawing WNT and assessed via morphology, ISC marker expression, and proliferation and differentiation status. To examine the ISC response to pathogenic insult, differentiated HIEs were infected with human RV. qRT-PCR was used to assess expression of LGR5, HOPX, and WNT target genes. To hone in on the ISC niche, we utilized FACS for CD44+ cells to isolate and specifically examine the proliferating compartment. We determined that undifferentiated and differentiated HIEs have distinct morphology, proliferation and differentiation status. Differentiation also results in a dramatic alteration of ISC marker expression, where LGR5 expression decreases and HOPX expression increases. Moreover, upon WNT addition or RV exposure, this differentiation phenotype is reversed: with an induction of LGR5 and other WNT target genes and a downregulation of HOPX. More importantly, through FACS, we show that LGR5 induction is specific for CD44+ population and, therefore, the ISC compartment. These experiments identify a plastic ISC niche in HIEs, which can be induced during epithelial damage such as RV infection. This research uses clinically relevant, human pathophysiology models to delineate ISC responses that are not currently well understood.

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Yvonne Jean Huang, MD (Poster: YPSA-14)
University of Michigan, Division of Pulmonary and Critical Care Medicine

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YPSA Poster Abstracts

YPSA-1

β-Arrestin mediates the Frank-Starling mechanism of cardiac contractility

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The Frank-Starling law is a physiologic phenomenon that describes an intrinsic property of heart muscle, where increased cardiac filling leads to enhanced performance. The mechanism for this phenomenon involves enhanced length-dependent myofilament Ca^{2+} sensitivity. However, the upstream molecular events that link cellular stretch to the length-dependent myofilament Ca^{2+} sensitivity are poorly understood. We tested whether angiotensin II type 1 receptors (AT1Rs) and the adaptor protein β-arrestin are involved in the Frank-Starling law.

The Frank-Starling law was tested by pressure-volume loop analyses on wild-type (n=27), β-arrestin 1 KO (n=15), β-arrestin 2 KO (n=18), and AT1R KO (n=6) mice. Each group was given a colloid solution to increase preload in order to measure the relationship between cardiac filling and performance. The effect of pharmacologic AT1R blockade on the Frank-Starling law was tested in a separate cohort of wild-type mice treated with saline (n=10), a conventional AT1R blocker (n=10), or a β-arrestin-biased AT1R ligand (n=6). Myofilament Ca^{2+} sensitivity was tested in muscle fiber preparations isolated from wild-type, β-arrestin-1, β-arrestin 2, and AT1R KO mice.

We found that wild-type mice increased stroke volume by 50-60% with volume loading, while no significant increase in stroke volume was noted in β-arrestin 1, β-arrestin 2, or AT1R KO mice. Wild-type mice pretreated with a conventional AT1R blocker were unable to enhance cardiac contractility with volume loading, while treatment with a β-arrestin-biased AT1R ligand preserved the increase in contractility with volume loading. Lastly, in skinned muscle fiber preparations, we found length-dependent myofilament Ca^{2+} sensitivity in the β-arrestin 1, β-arrestin 2, and AT1R KO mice to be impaired.

Our data identify β-arrestin 1, β-arrestin 2, and the AT1R as key regulatory molecules in the Frank-Starling law of the heart.

YPSA-2

Yolk sac-derived renin progenitors contribute to primitive B lymphopoiesis and are at-risk for neoplastic transformation

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Our lab previously generated a genetic mouse model of leukemia through conditional deletion of the Notch effector gene, RBP-J, specifically within renin-expressing cells. Affected mice develop precursor B cell leukemia with a unique $B200^{dim}CD19^{+}CD43^{+}$ phenotype. The cell of origin for this leukemia was found to be a novel renin-expressing hematopoietic progenitor with B lymphocyte pedigree, present within hematopoietic tissues. We have now conducted further studies on the cell of origin in this model, and we have detected renin-expressing cells within hematopoietic tissues during fetal life. Using *in vivo* lineage-tracing techniques, flow cytometry, immunofluorescence, *in situ* hybridization, and polymerase chain reaction (PCR) analysis, we found that renin-expressing hematopoietic progenitors first appear within the yolk sac during mid-gestation (E11.5). Subsequently, renin lineage cells expand from the yolk sac and colonize the fetal liver (E15.5), spleen (E15.5), and bone marrow (E16.5). Renin lineage cells within the hematopoietic system peak in number just prior to birth and then decrease with post-natal age except in the peritoneal cavity, where they persist throughout adult life as B-1 B cells. In the fetal liver and fetal spleen, the renin lineage cells express B cell surface markers including CD19 and CD43; however they have dim B220 expression – an identical phenotype as the B cell leukemia clones which occur with RBP-J deletion. Deletion of RBP-J within other B cell subsets, including CD19-expressing and Mb1-expressing B cells, does not result in leukemia. These studies suggest that renin progenitors appear during the initial wave of primitive B lymphopoiesis within the yolk sac and then expand to the fetal liver and spleen prior to the development of definitive hematopoiesis. Further, these novel progenitors appear to be uniquely vulnerable to leukemia transformation, thus establishing a fundamental link between embryonic and neoplastic lymphopoiesis.

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YPSA-3

Evidence of fatty acid metabolic defects and right ventricular lipotoxicity in human pulmonary arterial hypertension

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The mechanisms of right ventricular (RV) failure in pulmonary arterial hypertension (PAH) are poorly understood. Abnormalities in fatty acid (FA) metabolism have been described in experimental models of PAH, but systemic and myocardial FA metabolism have not been studied in human PAH. We hypothesize the FA metabolic defects are present in human PAH and contribute to RV lipotoxicity.

We used human blood, RV tissue, and non-invasive imaging to characterize multiple steps in the FA metabolic pathway in PAH subjects and controls. Circulating FFAs and long-chain acylcarnitines were elevated in PAH patients versus controls after adjusting for multiple comparisons (both $p < 0.001$). Human RV long-chain FAs were increased and long-chain acylcarnitines were reduced nearly 100-fold in PAH versus controls ($p < 0.001$). Using proton magnetic resonance spectroscopy to measure *in vivo* intramyocyte lipid, we found 7-fold higher triglyceride content in human PAH versus controls (1.4 ± 1.3 %TG vs. 0.22 ± 0.11 %TG, $p = 0.02$). Ceramide, a mediator of lipotoxicity, was increased in PAH RVs versus controls ($p = 0.006$). Using an animal model of heritable PAH we demonstrated reduced fatty acid oxidation via failure of palmitoylcarnitine to stimulate oxygen consumption in the PAH RV ($p < 0.001$).

Abnormalities in fatty acid metabolism can be detected in the blood and myocardium in human PAH and are associated with cardiac steatosis and lipotoxicity. Murine data suggest that lipotoxicity may arise from impaired fatty acid oxidation. This study highlights specific metabolic pathways of potential therapeutic interest in PAH and establishes a tool to study their activity *in vivo*. Further studies are needed to determine the functional significance of these findings and whether metabolic therapies targeting fatty acid oxidation can favorably modify RV metabolism in PAH.

YPSA-4

Exploring mechanisms of ganetespib resistance in KRAS mutant NSCLC

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Approximately 25% of non-small cell lung cancer (NSCLC) patients have *KRAS* mutations, and no current therapies targeting this oncogenic driver exist. Heat shock protein 90 (HSP90) is a molecular chaperone required for the stability of "client" oncoproteins, many of which are *KRAS* effectors. Unfortunately, only transient responses were observed in early clinical studies of single-agent HSP90 inhibitors (HSP90i) in *KRAS* mutant NSCLC. It is critical to understand the mechanisms of acquired resistance to these inhibitors in order to develop rationale and efficacious combinations. Therefore, we examined the mechanism(s) of acquired resistance to ganetespib, an HSP90i currently in phase III clinical trials.

We have generated ganetespib-resistant (GR), *KRAS* mutant NSCLC cells and demonstrated that bypass of G2/M arrest and hyperactivation of RAF/MEK/ERK and PI3K/AKT/MTOR pathways contribute to resistance. We observed increased dependence on these two pathways as GR cells were more sensitive to ERK1/2 or dual PI3K/MTOR inhibition. Moreover, the combination of ganetespib with ERK1/2 or PI3K/MTOR inhibitors was more effective than either single agent alone. Interestingly, the expression and activity of the serine/threonine kinases, p90 ribosomal S6 kinases (p90-RSK), important regulators of G2/M progression and the PI3K/AKT/MTOR pathway and key targets of ERK1/2 and PDK1, were significantly increased. We observed that isoform-specific genetic silencing and pharmacological inhibition of p90-RSK re-sensitized GR cells to ganetespib. Conversely, overexpression of distinct p90-RSK isoforms in sensitive cell lines induced ganetespib resistance and led to bypass of ganetespib-induced G2/M arrest. The combination of ganetespib with p90-RSK inhibitor(s) enhanced cytotoxicity compared with either agent alone. These data suggest that combination of ganetespib with inhibitors specifically targeting ERK1/2, dual PI3K/MTOR, or p90-RSK may prevent resistance and/or overcome acquired resistance after ganetespib treatment, providing the preclinical rationale for our planned phase I trial of the combination of ganetespib and a dual PI3K/MTOR inhibitor in *KRAS* mutant NSCLC.

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YPSA-5

Genomic landscape of cutaneous T cell lymphoma

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Cutaneous T cell lymphoma (CTCL) is an incurable non-Hodgkin lymphoma of skin-homing T lymphocytes. We performed exome and whole genome DNA sequencing and RNA sequencing on purified CTCL and matched normal cells. We systematically identified the somatic copy number variants (SCNVs) and somatic single nucleotide variants (SSNVs) in each tumor. Using an integrative analysis, we systematically identified driver genes in CTCL, including genes involved in T cell activation and apoptosis, NFκB signaling, chromatin remodeling, DNA damage response, JAK-STAT signaling pathway, and T cell receptor signaling. We also identified recurrent, gain-of-function mutations in CD28's extracellular domain (p.F51V and p.Q77P), which were unlikely to occur by chance alone. CD28 is the canonical costimulatory molecule whose activation is critical for optimal T cell receptor activation. These mutations increase its avidity for its ligand CD86 and potentiate ligand-dependent CD28 signaling. Strikingly, somatic copy number variants (SCNVs) comprise 92% of all driver mutations (mean of 11.8 pathogenic SCNVs vs. 1.0 somatic single nucleotide variants per CTCL). For example, the relative contribution of focal deletions to CTCL is significantly higher than for all cancers sequenced as part of the cancer genome atlas (TCGA), establishing CTCL as an outlier in cancer. We hypothesized that the frequent focal deletions were the result of RAG endonucleases. These enzymes are normally expressed in developing thymocytes and catalyze T cell receptor gene assembly. To elucidate the mechanisms underlying structural variants in CTCL, we performed whole genome sequencing (WGS), which revealed a statistically significant enrichment of putative RAG binding sites at CTCL breakpoints. These data suggest that structural variants may have occurred during T cell maturation in the thymus. Collectively, our findings have advanced our understanding of CTCL pathogenesis and led to the discovery of potential therapeutic targets.

YPSA-6

Brca1 deficiency causes bone marrow failure and spontaneous hematologic malignancies in mice

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BRCA1 is critical for maintenance of genomic stability and interacts directly with several proteins that regulate hematopoietic stem cell function and are part of the Fanconi anemia (FA) double-strand break DNA repair pathway. The effects of complete BRCA1 deficiency on bone marrow function are unknown. To test the hypothesis that Brca1 is essential in hematopoiesis, we developed a conditional mouse model with Mx1-Cre-mediated Brca1 deletion. Mice lacking Brca1 in the bone marrow have baseline cytopenias and develop spontaneous bone marrow failure (BMF) or diverse hematologic malignancies by six months of age. Brca1$^{-/-}$ bone marrow cells have a reduced capacity to form hematopoietic colonies *in vitro* and to reconstitute hematopoiesis in irradiated recipients, consistent with a hematopoietic progenitor functional defect. Brca1$^{-/-}$ BM cells also show FA-like hypersensitivity to the DNA cross-linking agent, mitomycin C, and karyotypes feature genomic instability. Taken together, our results show that loss of Brca1 in murine bone marrow causes hematopoietic defects similar to that seen in people with FA, providing strong evidence that Brca1 is critical for normal hematopoiesis and that Brca1 is a bona fide FA-like gene.

YPSA-7

Increasing preosteoclast secretion of platelet-derived growth factor type BB prevents glucocorticoid-induced osteoporosis by stimulating angiogenesis and osteogenesis

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Survival of chronic diseases in childhood is often achieved utilizing glucocorticoids. However, the survival comes at a cost to the growing skeleton, resulting in secondary osteoporosis. We recently found that preosteoclasts secrete platelet-derived growth factor type BB (PDGF-BB) to promote angiogenesis and osteogenesis. As glucocorticoid therapy affects both osteoclast bone resorption and osteoblast bone formation, we explored if loss of osteoclast cathepsin K activity could increase secretion

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of PDGF-BB by preosteoclasts and therefore protect bone mass in young mice exposed to prednisolone. A glucocorticoid-induced mouse model was developed in young mice by treatment with prednisolone at 10 mg/m^2/day beginning at 2 weeks of life and continuing for 4 weeks. Global cathepsin K knockout (Ctsk$^{-/-}$) mice and wild-type littermates were treated with prednisolone or vehicle. Histological analysis with tartrate resistance acid phosphatase (Trap) staining and immunostaining for osteocalcin were analyzed. H-type vessels were identified by immunofluorescence staining for CD31 and endomucin. PDGF-BB serum concentrations were determined by ELISA. Microcomputed tomography was analyzed to understand global effects on bone. Trap staining demonstrated that osteoclast numbers decrease in response to prednisolone, whereas loss of cathepsin K ameliorated this decrease largely by increasing Trap$^+$/PDGF-BB$^+$ cells in the bone marrow. Serum concentrations of PDGF-BB decreased in wild-type mice but increased in Ctsk$^{-/-}$ treated with prednisolone compared to Ctsk$^{-/-}$ vehicle controls. The decreased angiogenesis and osteogenesis, as assessed by H-type vessels and osteocalcin staining, observed in wild-type mice treated with prednisolone were attenuated in Ctsk$^{-/-}$ mice treated with prednisolone. Overall, the osteoporotic phenotype assessed by μCT noted in wild-type mice treated with prednisolone relative to vehicle treatment was prevented in Ctsk$^{-/-}$ mice treated with prednisolone relative to vehicle. These preliminary data suggest that cathepsin K inhibition may prevent glucocorticoid-induced osteoporosis in young mice by increasing preosteoclast secretion of PDGF-BB.

YPSA-8

Whole exome sequencing reveals *carboxylesterase 1* as a novel gene in methamphetamine-induced pulmonary arterial hypertension

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Pulmonary arterial hypertension is a known complication of methamphetamine use (METH PAH), but whether there are genetic factors involved in disease susceptibility is unknown. In this study, we used whole exome sequencing (WES) to screen 18 METH PAH patients for high-risk mutations.

WES followed by ANNOVAR analysis identified 147,360 high-risk variants in 1,767 genes of which 12 were chosen for further study. Our top candidate was *carboxylesterase 1 (CES1)*, a gene coding for an enzyme responsible for detoxification of stimulants such as cocaine and methylphenidate. About 95% of METH PAH patients were heterozygous carriers of a single nucleotide variant (SNV, rs115629050) predicted to reduce CES1 activity compared to wild type alleles. Since immunofluorescence studies of human lung tissue demonstrated that CES1 is predominantly expressed in the pulmonary endothelium, we assessed the effect of wild-type and mutant CES1 in protecting human pulmonary microvascular endothelial cells (PMVECs) against METH-induced damage. Compared to the empty vector, PMVECs transfected with the mutant CES1 demonstrated significantly higher rates of METH induced apoptosis, whereas the opposite was found in cells transfected with the wild-type construct. The mechanism for METH-induced toxicity appears to be associated with internalization and metabolism of METH by PMVECs followed by increased formation of reactive oxygen species (ROS) and compensatory autophagy. Compared to healthy controls, CES1-deficient PMVECs lack a robust autophagy response despite higher levels of ROS, which correlate with increased METH-induced apoptosis.

WES analysis led to the discovery of *CES1* as a novel candidate gene in METH PAH. Mutations that reduce CES1 activity could promote development of METH PAH by inducing apoptosis and progressive loss of pulmonary microvessels. Screening for CES1 mutations could help identify high-risk active and former METH users and facilitate early diagnosis of PAH.

YPSA-9

Elevated circulating thymic stromal lymphopoietin suppresses breast cancer development

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Breast cancer is the second most common type of cancer among women in the United States, affecting 12.4% of women with 40,000 deaths estimated each year. Epidemiological data suggest that individuals with the history of allergic diseases like atopic dermatitis and asthma have reduced risk of developing breast cancer. However, a mechanistic link between atopic disease and breast cancer protection is lacking. We suspected that thymic stromal lymphopoietin (TSLP) may be relevant because it is an epithelial-derived cytokine that is a master regulator of allergic inflammation in the skin and lung. Moreover, we previously showed that epidermal-derived TSLP blocks skin cancer. We also demonstrated that TSLP released from the skin can reach high levels in the systemic circulation and cause allergic inflammation at distant organs. Therefore, we hypothesized that skin-derived TSLP can impact breast carcinogenesis by inducing an anti-tumor immunity in the breast. Herein, we used mice with spontaneous breast cancer and crossed them to animals overexpressing TSLP in their skin. Interestingly, we found that overexpression of TSLP in the skin

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and its high circulating levels led to significant resistance against breast cancer development. The tumors in mice with TSLP were largely arrested in the early adenoma-like stages, and these mice were protected from developing metastatic disease. We observed accumulation of activated $CD4^+$ T helper 2 (Th2) cells in the breast tumors and their draining lymph nodes in the mice with TSLP as compared to animals without TSLP. Importantly, we found TSLP-stimulated Th2 cells to be both required and sufficient to establish a robust tumor immunity against breast cancer. Finally, we administered an FDA-approved topical agent (calcipotriol), which induces TSLP in the skin, and showed that it prevented breast tumor growth. Together, our findings establish a mechanistic explanation for the reduced risk of breast cancer in atopic patients and a critical role for TSLP in anti-tumor immunity in the breast, suggesting that TSLP induction may be therapeutically beneficial.

YPSA-10

Risk of incident hypertension and chronic kidney disease following shock wave lithotripsy and ureteroscopy: a population-based cohort study using The Health Improvement Network

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We sought to determine if among individuals with urolithiasis, extracorporeal shock wave lithotripsy (SWL) and ureteroscopy (URS) are associated with a higher risk of incident hypertension (HTN) and/or chronic kidney disease (CKD). A population-based retrospective cohort study using The Health Improvement Network comprised 11,570 participants with incident urolithiasis and 127,464 without urolithiasis; pre-existing HTN and CKD were excluded. 1,319 and 919 of urolithiasis participants had ≥1 SWL or URS procedure, respectively. Multivariable Cox regression was used to estimate the hazard ratio (HR) for incident CKD stage 3-5 and HTN in separate analyses. Over a median of 3.7 and 4.1 years, 1,423 (12.3%) and 595 (5.1%) of urolithiasis participants developed HTN and CKD, respectively. Urolithiasis was associated with a HR for HTN of 1.42 (95% CI: 1.35, 1.51; $p<0.001$) and for CKD of 1.82 (95% CI: 1.67, 1.98; $p<0.001$). SWL was associated with an increased risk of HTN (HR 1.34, 95% CI: 1.15, 1.57; $p<0.001$), while URS was not. When further stratified as SWL to the kidney or ureter, only SWL to the kidney was independently associated with HTN (HR 1.40, 95% CI: 1.19, 1.66; $p<0.001$). Neither SWL nor URS was associated with incident CKD. Given that urolithiasis itself was associated with a HR of 1.42 for HTN, an individual who undergoes SWL to the kidney can be expected to have a HR of 1.96 (95% CI: 1.67, 2.29; $p<0.001$) compared to an individual without urolithiasis.

YPSA-11

Hematopoietic stem cell-based discovery of critical host erythrocyte determinants of malaria infection

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Severe malaria caused by the protozoan parasite *Plasmodium falciparum* is a major cause of childhood mortality, particularly in resource-poor settings. During clinical disease, parasites invade and remodel erythrocytes, leading to microvascular congestion and organ failure. Invasion of erythrocytes by *P. falciparum* requires contributions from both the parasite and the host cell. Knowledge of host determinants of infection could guide the development of new therapeutics for malaria, but the lack of a nucleus in mature red blood cells has precluded their genetic analysis. We recently reported a hematopoietic stem cell-based forward genetic screen to identify human blood group genes required for *P. falciparum* invasion. We found that the top candidate, CD55 (DAF), is essential for parasites to attach to the erythrocyte plasma membrane during invasion. Another top candidate was the ATP binding cassette transporter *ABCB6,* which encodes the Lan blood group. ABCB6 is localized to the plasma membrane in erythrocytes, where it has an unknown function. Using peripheral red blood cells from several unrelated Lan-null individuals and controls, we observed functionally that *P. falciparum* requires ABCB6 for efficient invasion. The reliance of *P. falciparum* on ABCB6 was strain-transcendent, including multiple laboratory-adapted strains and clinical isolates. Notably, P. falciparum invaded ABCG2-null cells normally, suggesting that the phenotype in the Lan-null cells was not due to porphyrin accumulation. Further, the zoonotic parasite *P. knowlesi* invaded Lan-null and control cells with similar efficiency, suggesting that ABCB6 may specifically mediate *P. falciparum* invasion. We used tandem mass tag mass spectrometry to quantify all of the plasma membrane proteins on the Lan-null and control cells, and found that the only consistent difference was ABCB6. These findings suggest that ABCB6 is a critical host determinant for all strains of *P. falciparum*, and may interact with a parasite ligand that could serve as an important vaccine candidate.

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YPSA-12

Macrophage-specific mineralocorticoid receptor antagonists for heart failure

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Heart failure (HF) is a major cause of morbidity and mortality in the world. Mineralocorticoid receptor (MR) activation is associated with oxidative stress, inflammation, and an increased risk of HF. Although MR antagonists (MRA) have been used in HF patients and consistently improve outcomes, the mechanisms underlying these beneficial effects still remain largely unknown. Recent studies have suggested that macrophage MR activation coordinates inflammatory responses and may contribute to HF initiation and that chemical ablation of macrophage MR may be beneficial in HF.

To further understand the mechanisms through which macrophage-MR drives inflammation and pressure-overload HF, we engineered a macrophage-specific MRA (Mφ-MRA) using eplerenone (an MRA) and a dendrimer nanoparticle and evaluated its biological activity *in vitro* and *in vivo*.

Bone marrow-derived macrophages (BMDM) and control cell models were used to evaluate the cell-specificity and therapeutic efficacy of the Mφ-MRA. Using flow cytometry, we found that the Mφ-MRA internalizes and is activated specifically inside macrophages and not other cell types. In response to aldosterone (Aldo), the ligand for the MR, BMDMs had increased oxidative stress which was abrogated in a dose-responsive manner by the Mφ-MRA. Additionally, the Mϕ-MRA blunted TNFα induction after Aldo treatment of BMDMs. Finally, preliminary studies in a murine model of pressure-overload HF demonstrated that the Mφ-MRA had a greater reduction in fibrosis compared with control mice (7.5% vs. 2.5%; $P < 0.05$).

We have developed a novel, macrophage-specific MRA using a synthetic nanoparticle and an established MRA to understand the contribution of macrophage-MR signaling to the pathobiology of HF. Our preliminary studies demonstrate that the Mφ-MRA is biologically active *in vitro* and *in vivo* and slows HF progression suggesting that macrophage-MR signaling is a critical driver of systemic inflammation and HF.

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YPSA-13

Pharmacogenomic dissection of mTOR-mediated mRNA translation in cancer and drug resistance

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Deregulation of translation control is a fundamental mechanism that can reprogram cells towards tumorigenesis and cancer progression. mTOR (mammalian target of rapamycin) is a master regulator of mRNA translation that links post-transcriptional regulation directly with many cellular functions important in cancer initiation and progression including cell growth, cell proliferation, and metabolism. However, the molecular mechanism by which upstream signal transduction pathways interface with the translation machinery to direct cancer pathogenesis remains poorly defined. To directly address this question, we conducted a genome-wide ribosomal profiling study in human prostate cancer cells upon differential mTOR inhibition. Optimizing this technology to determine the translational landscape of human prostate cancer, we surprisingly discovered a cohort of mRNAs translationally regulated by oncogenic mTOR signaling which fundamentally alters epithelial cell behavior towards invasion and metastasis. In particular, we will discuss the mechanism by which specific transcripts are deployed for translation in response to eukaryotic initiation factor 4E (eIF4E) hyperactivity downstream of oncogenic mTOR signaling. Moreover, we will show that pharmacological inhibition of the mTOR kinase with novel ATP active site inhibitors is detrimental for cancer progression and that inhibition of a specific translational node, the 4EBP1/eIF4E axis, downstream of oncogenic mTOR is required for therapeutic efficacy. Lastly, utilizing a new mouse model to toggle protein synthesis *in vivo*, we demonstrate that cell type-specific protein synthesis rates determine sensitivity or intrinsic resistance to mTOR kinase inhibitors. Together, our findings show that translation control of highly specific nodes of gene expression provides a rapid and druggable mechanism that directs specific cancer phenotypes, as well as a cell type-specific mechanism for drug resistance.

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YPSA-14

Airway microbial communities can subsist on inhaled corticosteroids and associate with diminished response to inhaled corticosteroids in adult asthma

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Few treatment alternatives exist for the >30% of asthmatic patients who do not respond to inhaled corticosteroids (ICS). The lower airway microbiome differs in asthmatic versus healthy adults and may shape asthma phenotypic features including responsiveness to ICS. In moderate asthma sub-optimally controlled by ICS, we previously reported significantly relationships between specific bronchial bacteria and greater airway hyper-reactivity. To further understand microbial contributions to ICS response, we studied severe asthma patients requiring high-dose ICS and examined the effects of ICS exposure on bacterial species associated with poorly controlled asthma.

The bronchial microbiome of 30 severe asthma patients was studied by molecular methods and compared to that of moderate asthma (n=41) and healthy subjects (n=7). Ecology-based statistics were used to test associations between microbiota composition, asthma control, and epithelial expression of genes reflective of steroid-response, among other features. Bacterial isolates from asthmatic and non-asthmatic patients *(Stenotrophomonas maltophilia, Klebsiella pneumoniae)* were cultured at 37°C up to two weeks in baffled flasks containing minimal media supplemented with fluticasone propionate (FP) only (200μM, 100μM, or 50μM).

Significant differences in the bronchial microbiome exist between severe and moderate asthma. In severe asthma, worsening of asthma control correlated (R=0.5-0.7, Benjamini-Hochberg $p_{adj}<0.05$) with greater bronchial abundance of Proteobacteria, representative of many potential pathogens including *S. maltophilia* and *K. pneumoniae*. Proteobacteria abundance also correlated with lower epithelial expression of FKBP5, a steroid-responsive gene. Both *S. maltophilia* and *K. pneumoniae* demonstrated growth and sustained viability in the presence of FP compared to minimal media alone (up to a 6-log difference in colony-forming units/mL). FP concentrations measured by LC-MS/MS in non-inoculated media showed relative stability over 1 week at 37°C.

Potentially pathogenic members of the airway microbiome in poorly controlled asthma can subsist on inhaled corticosteroids, suggesting microbial mechanisms may shape perceived ICS-responsiveness in asthma.

YPSA-15

Unraveling complex rearrangements in cancer with linked-reads

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Cancer genomes are rewired by rounds of DNA breakage and fusion, which yield rearranged loci and copy number alterations. While standard-library Illumina-whole genome-sequencing (WGS) enables some fine-mapping of aberrant DNA junctions and quantification of copy number, it provides limited insight into the long-range contiguity of rearranged fragments. Insight into long-range structure is essential to predict the functional impact of complex cancer rearrangements, e.g., the creation of neomorphic fusion proteins or the apposition of regulatory elements with oncogenes or tumor suppressors. Such insight also is crucial to inferring the somatic evolutionary histories of rearranged loci and characterizing novel mutational processes.

Combining standard library Illumina WGS with the recently developed 10X Linked-Read technology, we are able to gain significant insight into the long-range genome structure of a highly rearranged and aneuploid cell line (HCC1143). Using "GemCode"-based WGS libraries sequenced to 60-fold average coverage, we demonstrate long-range scaffolding of aberrant DNA junctions and reconstruction of highly rearranged loci at the 10-100kbp scale. Several of the recurrent structures in this genome suggest a novel rearrangement event that combines "classic" long-range fusion or fold-back inversion junctions with insertion of one or more genomic fragments ranging in size between 100bp and 20kbp. The majority of long-range rearrangement patterns consistent with this signature (particularly those beyond 1-2 kB) can not be unambiguously resolved using mate-pair or standard insert-size Illumina libraries. Our analysis demonstrates how 10X Linked-Reads can be used to infer the *cis* structure of highly rearranged cancer loci and gain insight into mutational processes that shape the cancer genome.

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YPSA-16

Myocardial NF-κB activation is essential for zebrafish heart regeneration

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Heart regeneration offers a novel therapeutic strategy for heart failure. Unlike mammals, lower vertebrates such as zebrafish mount a strong regenerative response following cardiac injury. A better understanding of the mechanisms that contribute to zebrafish heart regeneration can instruct approaches to achieve therapeutic heart regeneration in humans. Heart regeneration in zebrafish occurs by cardiomyocyte proliferation and reactivation of a cardiac developmental program, as evidenced by induction of *gata4* regulatory sequences in regenerating cardiomyocytes. While many of the cellular determinants for heart regeneration have been elucidated, how injury triggers a regenerative program through dedifferentiation and epicardial activation is a critical outstanding question. Here, we show that NF-κB signaling is induced in cardiomyocytes following injury. Expression profiling of regenerating zebrafish hearts shows increased levels for members of the NF-κB pathway in regenerating hearts. Using a transgenic reporter strain for NF-κB activity, we found NF-κB to be induced in cardiomyocytes following injury. We subsequently developed a transgenic zebrafish strain to conditionally inhibit NF-κB signaling in cardiomyocytes by expression of mutant IκBα. Myocardial inhibition of NF-κB activity blocks heart regeneration with pleiotropic effects, decreasing both cardiomyocyte proliferation and epicardial responses. Activation of gata4 regulatory sequences is also prevented by NF-κB signaling antagonism, suggesting an underlying defect in cardiomyocyte dedifferentiation. Our results implicate NF-κB signaling as a key node between cardiac injury and tissue regeneration.

YPSA-17

Dietary sodium and potassium intake, immune tolerance, and risk of Crohn's disease and ulcerative colitis

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Recent animal studies have identified that dietary salt intake may modify the risk and progression of autoimmune disorders through modulation of the IL23/T_H17 pathway, which is critical in the pathogenesis of ulcerative colitis (UC) and Crohn's disease (CD). We examined the association between dietary intake of sodium and potassium in relation to the T_H17 pathway on subsequent risk of incident UC and CD.

We conducted a prospective study of U.S. women enrolled in the Nurses' Health Study (NHS) and NHSII who provided detailed and validated information on diet and lifestyle beginning in 1984 in NHS and 1991 in NHSII. We confirmed incident cases of UC and CD reported through 2010 in NHS and 2011 in NHSII. We used Cox proportional hazards models to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) adjusted for potential confounders. In a case-control study nested within these cohorts, we evaluated the interaction between single nucleotide polymorphisms (SNPs) in genes involved in T_H17 pathway and dietary potassium on risk of CD and UC. In a cohort of healthy volunteers, we also assessed the effect of supplemental potassium on development of naïve and memory T cells, differentiated with TGFβ1 or T_H17 conditions.

Among a total of 194,711 women over a follow-up of 3,220,247 person-years, we documented 273 cases of CD and 335 cases of UC. Dietary intake of potassium (P_{trend} = 0.005) but not sodium (P_{trend} = 0.44) was inversely associated with risk of CD. Dietary potassium (P_{trend} = 0.08) but not sodium (P_{trend} = 0.77) was non-significantly inversely associated with risk of UC. Loci involved in the T_H17 pathway that have previously been associated with susceptibility to CD, particularly SNP rs7657746 *(IL21),* appeared to modify the association of potassium with risk of CD ($P_{interaction}$ = 0.004) and UC ($P_{interaction}$ = 0.01). *In vitro,* potassium enhanced the expression of *Foxp3* in both naïve and memory $CD4^+$ T cells and reinforced the expression of *Foxp3* in T_H17 cells even in the presence of a pro-inflammatory environment. In addition, potassium not only suppresses the expression of *Smad7* but also enhances both phosphorylation and expression of *Smad2* and *Smad3* offering a potential pathway by which potassium enhances *Foxp3* expression. Finally, potassium induces *IL10* expression in iTreg, further supporting a role for potassium in maintaining mucosal tolerance.

In this large prospective cohort study, dietary intake of potassium was associated with lower risk of CD and UC. The risk appeared to differ according to genetic variation at CD and UC susceptibility loci associated with the T_H17 pathway. These results are supported by functional studies demonstrating that *in vitro* treatment of T cells with potassium enhanced the expression of *Foxp3* by suppressing *Smad7* and enhancing *Smad2/3* functions.

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YPSA-18

Next-generation sequencing of *Alu* expression in dry age-related macular degeneration

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Age-related macular degeneration (AMD) is the most common cause of irreversible blindness in the developed world. We have previously demonstrated increased levels of non-coding RNA derived from *Alu* sequences in human eyes with advanced dry AMD (geographic atrophy, GA) due to loss of the RNA processing enzyme DICER1 using multiple techniques including adaptor-ligation PCR and in-situ immuno-localization. This accumulation of *Alu* RNA is toxic to the retinal pigment epithelium (RPE), a monolayer that is critical for the maintenance of overlying photoreceptors and optimal vision. In this study, we developed a next-generation *Alu* RNA sequencing (*Alu*-Seq) pipeline to quantify RNA copy numbers of all known *Alu* sub-family sequences in complex biological samples and map their origins in the human genome. We found increased *Alu* RNA levels in an *in vitro* model of *Dicer1* deficiency and in GA eyes. These techniques allowed us to identify a specific pattern of *Alu* subfamily gene expression with significant up-regulation of *Alu* Y RNA compared to others. Size fractionation of input RNA resulted in improved detection of elevated *Alu* RNA expression but was not required for subfamily assignment or quantification. Utilizing advanced bioinformatics methods, *Alu* expression was partitioned into transcriptomic and inter-genic regions. Specific loci of *Alu* inserts in the human genome were identified as robust transcriptional sites for aberrant expression that may be important in the etiology and risk of AMD progression. These data reveal a novel signature of pathogenic *Alu* subfamily expression, reveal genomic hotspots for transcribed *Alu*, and provide critical insight into the development of bioinformatics pipelines for *Alu* sequencing data.

YPSA-19

Epigenetic mechanisms of drug resistance in leukemia

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Resistance to therapy presents one of the major challenges in cancer treatment today, and novel approaches to identify and overcome the mechanisms that cause resistance are desperately needed for improving outcome. Acute T-cell lymphoblastic leukemia (T-ALL) is an aggressive hematopoietic malignancy in children and young adults that frequently becomes treatment-refractory and relapses. NOTCH[1] is a key oncogenic driver in T-ALL, and is aberrantly activated in more than 50% of the cases. Many therapies targeting the Notch pathway, including gamma secretase inhibitors (GSI), have been developed and tested. Yet, the rapid development of resistance that occurs with Notch inhibition *in vivo* has so far prevented the translation of these inhibitors into the clinical setting. Over the past years, we have developed a model of therapeutic resistance to Notch inhibition in T-ALL that is mediated through epigenetic state transitions, thus establishing epigenetic resistance mechanisms in leukemia for the first time. Rare GSI-tolerant "persister" cells are already present in the naïve T-ALL population – existing in dynamic equilibrium with GSI-sensitive cells – and give rise to the GSI-resistant population after prolonged treatment with GSI (Compound E). Persister cells have several unique dependencies that distinguish them from GSI-sensitive cells: ([1]) Persisters require compensatory oncogenic signaling through the TOR/ AKT pathway. ([2]) Persisters exhibit an altered epigenetic state consistent with both global chromatin compaction and local changes at enhancers of genes that are critically important for cell survival and lineage-defining genes. In this context, persister cells are exquisitely sensitive to BET inhibition, suggesting the potential to use drugs targeting enhancer states in combination therapy for acute lymphoblastic leukemia.

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YPSA-20

Identification of isoform-specific histone deacetylase inhibitors that potently reactivate the human immunodeficiency virus from latency

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Approximately 37 million people are afflicted with human immunodeficiency virus (HIV) with over 1.2 million deaths annually. Although anti-retroviral therapy (ART) can suppress the virus, it does not provide cure. The main obstacle to HIV eradication from patients on ART is viral latency in resting CD4-positive T cells. One approach to eliminate the viral reservoir is the "shock and kill" strategy, which involves the use of small-molecule latency reversing agents (LRAs) to stimulate viral replication in resting T cells in patients on ART. Viral replication will then result in death of infected T-cells thereby eliminating the reservoir. Histone deacetylase (HDAC) inhibitors are the leading candidate LRAs with vorinostat and others currently in pre-clinical testing. However, pan-HDACs like vorinostat may be less potent or have more side effects making isoform-specific HDACS more desirable.

Using the JLAT10.6 cell lines, we screened 15 isoform-specific HDAC inhibitors in our library for the ability to reactivate HIV from latency. Three compounds, SDL148, JMF1080 and SDL256 - largazole and two analogs - were found comparable to, or more potent than, vorinostat in reactivating HIV from latency. Unlike vorinostat, these compounds specifically inhibited class I HDACs as measured by levels of histone H3 acetylation in Western blot assays. In addition, one of the compounds, SDL148, was 20-times more potent at inhibiting class I HDAC compared to vorinostat. Next, using the Greene primary cell model of HIV latency, the largazoles were able to reactivate HIV from latency in nanomolar concentrations. Moreover, in resting T-cells isolated from virologically suppressed patients, SDL148 was able to reactivate virus from latency as determined by reverse transcriptase polymerase chain reaction and HIV-1 p24 measurements in culture supernatants. Thus, largazole and its analogs hold potential as novel isoform-specific HDAC inhibitors that can reactivate HIV from latency, and may help in the HIV eradication efforts.

YPSA-21

Quantitative clonal dynamics define mechanisms for CLL evolution in response to combination chemotherapy

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Clonal evolution is a central feature of chronic lymphocytic leukemia (CLL) relapse (Landau et al, Cell, 2013; Nature, 2015). This raises a fundamental question in cancer biology: what enables the relapse clone to replace the pre-treatment clone? In other words, is the increased fitness of the relapse clone due to a *lower death rate during therapy or a higher growth rate following therapy?*

To address this question we quantified clone-specific death and growth rates by targeted deep sequencing of frequent serial peripheral blood samples, beginning at pre-treatment and ending at relapse. We discerned different mechanisms of relapse after first-line chemo-immunotherapy based on whether the relapse clone harbored mutated *TP53* (TP53mut) or other mutations. In CLLs where the relapse clone contained TP53mut (n=10), the TP53mut clone showed a lower death rate during therapy compared with the pre-treatment TP53 wild-type (TP53wt) clone (2.4 and 3.8 median $\log_{10}$ reduction, respectively; P = 0.02). On the other hand, the TP53mut clone showed only modestly higher growth rates during repopulation compared with the TP53wt clone (median growth rate of 0.8%/day vs. 0.6%/day, P = 0.13). Thus, differential sensitivity to therapy plays a primary role in TP53mut clonal evolution. In contrast, in the remaining cases (n = 8) whose relapse clone harbored mutations other than in *TP53 (e.g., NOTCH1, ATM, SF3B1)*, we did not find differential sensitivity ($\log_{10}$ reduction of 3.9 vs. 3.8, for the pre-treatment and relapse clones, respectively, P=0.9). The primary engine leading to clonal takeover was a 1.5-fold higher growth rate during repopulation of the relapse clone.

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These data uncover evolutionary mechanisms in a personalized fashion. Thus, precise quantitation of clone-specific fitness in the context of therapy provides the required knowledge infrastructure to design the next generation of therapeutic algorithms, to maximize overall tumor elimination, instead of merely selecting one clone over another.

YPSA-22

Loss of nuclear TDP-43 leads to nuclear RNA dysregulation in amyotrophic lateral sclerosis and frontotemporal degeneration human brain tissue

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Amyotrophic lateral sclerosis (ALS) and frontotemporal degeneration (FTD) are two fatal neurodegenerative diseases which share a common molecular neuropathology, the loss of nuclear TAR DNA binding protein 43 (TDP-43) due to sequestration of TDP-43 protein within cytoplasmic inclusions. TDP-43 protein is a heterogeneous nuclear ribonucleoprotein which binds RNA and affects RNA stability and splicing. The loss of nuclear TDP-43 is hypothesized to contribute to neurotoxicity due to a loss of normal nuclear function. To determine the changes in nuclear RNA metabolism associated with the loss of nuclear TDP-43 protein, we have developed a novel method of fractionating pathologic human brain tissue to isolate neuronal nuclei with or without TDP-43 protein for RNA sequencing. Analysis of over 1.6 billion uniquely mapped reads from 7 pathologic and 6 control human brain specimens revealed widespread transcriptomic alterations including significant differential expression of 5,576 genes, demonstration that loss of TDP-43 protein preferentially affects neurons in the upper neocortical layers, and altered auto-regulation of the gene encoding TDP-43 (*TARDBP)*. The loss of TDP-43 was also associated with differential gene element usage, in particular affecting levels of intronic and 3′ untranslated region RNA segments. These non-coding RNA segments were highly enriched for TDP-43 and hnRNP protein binding sites. Similar changes were observed upon genetic knockout of *TARDBP* using CRISPR-Cas9 in HEK 293T cells. We propose a novel model where sequestration of hnRNP proteins by these non-coding RNA segments leads to attenuation of hnRNP activity. Ongoing experiments will test whether experimental manipulation of non-coding RNA segments leads to abnormal cross-regulation of hnRNP activity. These studies demonstrate the utility of using advanced molecular methods to analyze human post-mortem brain tissue in order to better understand the underlying molecular changes associated with disease, all the while revealing novel basic insights into the regulation of gene expression.

YPSA-23

An adipose to pancreatic islet axis regulating diabetes

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A hallmark of type 2 diabetes mellitus (T2DM) is the development of pancreatic β cell failure in the face of insulin resistance, resulting in relative insulinopenia and hyperglycemia. We show that the adipokine adipsin has a beneficial role in maintaining pancreatic β cell function. Animals genetically lacking adipsin have glucose intolerance due to insulinopenia; isolated islets from these mice have reduced glucose-stimulated insulin secretion. Replenishment of adipsin to diabetic mice treated hyperglycemia by boosting insulin secretion. We identify C3a, a peptide generated downstream of adipsin action, as a potent insulin secretagogue and show that the C3a receptor is required for these beneficial effects of adipsin. C3a acts on islets by augmenting intracellular ATP levels, respiration, and cytosolic free Ca^{2+}. Finally, we demonstrate that T2DM patients with β cell failure are deficient in adipsin. These findings indicate that the adipsin/C3a pathway connects adipocyte function to pancreatic β cell physiology and manipulation of this molecular switch may serve as a novel therapy in T2DM.

YPSA-24

Engineering human T cell circuitry

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Functional testing of human genome sequences in primary immune cells has been largely impossible until our advances in genome engineering methods that now permit direct DNA

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editing in human primary T cells. CRISPR/Cas9 has facilitated genome engineering in many cell types, but in human T cells Cas9 efficiency had been limited and Cas9 had not allowed targeted nucleotide replacements. We have now developed a CRISPR/Cas9-based platform that enables both knock-out and knock-in genome editing in primary human T cells by electroporation of Cas9:single-guide RNA ribonucleoproteins (Cas9 RNPs). Cas9 RNPs paired with homology-directed repair (HDR) template oligonucleotides can generate a high frequency of knock-in targeted genome modifications in primary T cells. The technology enables unprecedented explorations of genetic mechanisms that regulate T cell differentiation and function. We aim to understand how sequence variation throughout the human genome affects T cell circuits in health and disease. Cas9 RNP technology holds great potential for therapeutic genome engineering of human T cells for treatment of cancer, HIV, primary immune deficiencies, and autoimmune diseases.

YPSA-25

Muscle oxidative phosphorylation quantitation using creatine chemical exchange saturation transfer (CEST) MRI in mitochondrial disorders

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Systemic mitochondrial energy deficiency may contribute to the pathophysiology of many age-related human diseases. However, there is a paucity of *in vivo* biomarkers that reliably reflect the failure of oxidative phosphorylation (OXPHOS) in the presence of mitochondrial insufficiency. A particular problem is the absence of methods with adequate anatomic resolution to detect mitochondrial dysfunction in a tissue such as skeletal muscle, in which individual muscle groups vary widely with regard to their contractile and metabolic properties. To address this need, a non-invasive creatine chemical exchange saturation transfer (CrCEST) magnetic resonance imaging (MRI) technique has recently been developed that measures OXPHOS capacity in discrete muscle groups. In this proof-of-principle study, we found that individuals with genetic mitochondrial diseases and related disorders had prolonged post-exercise CrCEST recovery times, indicative of compromised OXPHOS capacity, in comparison with a cohort of healthy volunteers balanced for age, sex and body mass index. Our study population also demonstrated muscle group-specific differences in creatine metabolism. In summary, CrCEST provides a safe and accessible technique to non-invasively monitor dynamic changes of muscle mitochondrial function and creatine content with high anatomic resolution. Translational opportunities for CrCEST include use prior to muscle biopsy to improve diagnostic yield, and as a biomarker of therapeutic efficacy.

YPSA-26

The role of dermal Wnt activation in hair follicle development and carcinogenesis

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As a conserved regulator of growth, Wnt/β-catenin signaling plays a key role at the interface of development and cancer. During skin development, signals provided by the dermis are required for epithelial hair follicle induction and formation of the associated dermal papilla (DP), a dense cluster of dermal cells that regulates hair follicle growth. In adult skin, hair follicle stem cells that reside in the hair follicle epithelium drive hair follicle regeneration. We previously established that DP cells are critical for hair follicle stem cell activation and hair follicle regeneration. In this study, we developed a novel approach to investigate how DP cells regulate hair follicle growth during development and how they may influence growth of a common hair follicle tumor, basal cell carcinoma (BCC). Previous studies established that Wnt is active in the dermis and is required for hair follicle initiation. However, it is unknown (1) how the DP forms during development, (2) if DP cells originate from Wnt-activated dermal cells, and (3) how dermal Wnt regulates the formation or maintenance of the DP. Using live imaging of embryonic mouse skin explants coupled with genetic approaches, we show that DP cells originate from Wnt-activated dermal cells and that they form predominantly by cellular migration or compaction, while they show distinctively minimal proliferation. Inhibition of myosin IIB activation abrogates DP formation, suggesting that Wnt functions partly by regulating actomyosin dynamics. Further, we show that the tumor stroma of BCC lacks Wnt active dermal cells and that their growth is inhibited within the hair follicle where DP cells reside, suggesting that dermal Wnt activation may suppress BCC growth. Collectively, this study proposes a unifying mechanism to explain how dermal cells influence both normal and aberrant hair follicle growth and provides new avenues to promote tissue regeneration as well as to treat BCC.

YPSA Poster Abstracts

YPSA-27

Body fat distribution and incident cardiovascular disease in obese adults

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Differences in body fat distribution may underpin heterogeneity in cardiovascular disease (CVD) risk associated with obesity.

We aimed to explore the relative contributions of specific adipose tissue depots to CVD risk.

We analyzed data from obese (body mass index [BMI] ≥ 30 kg/m^2) participants in the Dallas Heart Study without baseline CVD who underwent dual energy x-ray absorptiometry and MRI assessment of adipose tissue distribution (visceral, abdominal subcutaneous, lower body subcutaneous, and liver) between 2000-2002. The composite outcome of incident and recurrent CVD events (cardiovascular death, non-fatal myocardial infarction, non-fatal stroke or transient ischemic attack, coronary or peripheral artery revascularization, heart failure hospitalization, atrial fibrillation hospitalization) was assessed over 9 years of follow-up. Associations were evaluated with multivariable-adjusted Cox models using the Wei-Lin-Weissfeld method for recurrent events. Sequential adjustment for other potential factors in the etiological pathway between adiposity and CVD was also performed.

The study cohort included 972 obese participants with mean age 44 years; 62% women; 54% African-Americans. 108 CVD events occurred in 81 participants. After adjustment for age, sex, race, hypertension, hypercholesterolemia, smoking, and BMI, visceral adipose tissue was positively associated with incident or recurrent CVD (hazard ratio [HR] per 1-standard deviation: 1.21 [95% CI 1.02, 1.43]), while lower body subcutaneous adipose tissue inversely associated with the composite outcome (HR per 1-standard deviation: 0.59 [95% CI 0.45, 0.76]). Associations of visceral, but not lower body, fat modestly attenuated after adjustment for diabetes status. No associations were observed for abdominal subcutaneous or liver fat.

Among obese adults, excess visceral fat associated with higher CVD risk, while increased lower body subcutaneous fat had a protective association. These findings highlight the biological importance of fat distribution with regard to CVD risk in obesity and suggest a potential prognostic role for imaging-based assessments of fat distribution.

YPSA-28

The risk of incident liver disease among patients with psoriasis, psoriatic arthritis, and rheumatoid arthritis

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Previous studies have suggested that psoriasis, psoriatic arthritis (PsA), and rheumatoid arthritis (RA) are associated with liver disease, but these studies have mainly been cross-sectional. The objective of this study was to examine the risk for incident liver disease among patients with psoriasis, PsA, and RA compared to the general population.

We conducted a population-based cohort study in the United Kingdom using The Health Improvement Network (THIN) data from 1994-2014. Patients age 18-89 with codes for PsA, RA, or psoriasis were included and were matched to up to 5 controls from the general population on practice and start date in the practice. Patients with liver disease were excluded. Cox proportional hazards models were used to estimate hazard ratios (HR) with 95% confidence intervals (CI). A priori we hypothesized an interaction with disease-modifying antirheumatic drug (DMARD) use. Patients with psoriasis who received a DMARD or phototherapy were identified as "severe" psoriasis.

Among patients with PsA (N=12,308), RA (N=54,251), psoriasis (N=197,130), and controls (N=1,279,754), 10,415 incident cases of liver disease were identified. After adjusting for age, sex, body mass index, drinking, smoking, NSAID use, hospitalization, hypertension, hyperlipidemia, and diabetes, the HR were significantly elevated for all groups except patients with RA who received a DMARD: PsA without a DMARD 1.39 (95%CI: 1.03-1.87), PsA with a DMARD 1.66 (95%CI: 1.28-2.14), RA without a DMARD 1.53 (1.29-1.80), RA patients with a DMARD 0.99 (0.85-1.15), psoriasis without a DMARD 1.38 (1.30-1.47), and severe psoriasis 1.91 (1.58-2.31).

Patients with PsA, RA, and psoriasis have a significantly increased risk for development of liver disease even after accounting for risk factors and among patients not prescribed a DMARD. These results suggest systemic inflammation may play a role in the development of liver disease beyond the relationship with treatment-related toxicity.

YPSA Poster Abstracts

YPSA-29

Hedgehog actively maintains adult lung quiescence and regulates repair and regeneration

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Postnatal tissue quiescence is thought to be a default state in the absence of a proliferative stimulus such as injury. Although previous studies have demonstrated that certain embryonic development programs are reactivated aberrantly in adult organs to drive repair and regeneration, it is not well understood how quiescence is maintained in organs such as the lung, which displays a remarkably low level of cellular turnover. Our studies demonstrate that quiescence in the adult lung is an actively maintained state and is regulated by hedgehog signaling. Epithelial-specific deletion of sonic hedgehog (*Shh*) during postnatal homeostasis in the murine lung results in a proliferative expansion of the adjacent lung mesenchyme. Hedgehog signaling is initially downregulated during the acute phase of epithelial injury as the mesenchyme proliferates in response, but returns to baseline during injury resolution as quiescence is restored. Activation of hedgehog during acute epithelial injury attenuates the proliferative expansion of the lung mesenchyme, whereas inactivation of hedgehog signaling prevents the restoration of quiescence during injury resolution. Finally, we show that hedgehog also regulates epithelial quiescence and regeneration in response to injury via a mesenchymal feedback mechanism. These results demonstrate that epithelial–mesenchymal interactions coordinated by hedgehog actively maintain postnatal tissue homeostasis, and deregulation of hedgehog during injury leads to aberrant repair and regeneration in the lung.

YPSA-30

Functional deficits precede structural lesions in mice with high-fat diet-induced diabetic retinopathy

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Obesity predisposes to human type 2 diabetes, the most common cause of diabetic retinopathy. To determine if high-fat diet-induced diabetes in mice can model retinal disease, we weaned mice to chow or a high-fat diet and tested the hypothesis that diet-induced metabolic disease promotes retinopathy. As compared to controls, mice fed a diet providing 42% of energy as fat developed obesity-related glucose intolerance by 6 months. There was no evidence of microvascular disease until 12 months, when trypsin digests and dye leakage assays showed high-fat-fed mice had greater atrophic capillaries, pericyte ghosts, and permeability than controls. However, electroretinographic dysfunction began at 6 months in high-fat-fed mice, manifested by increased latencies and reduced amplitudes of oscillatory potentials compared to controls. These electroretinographic abnormalities were correlated with glucose intolerance. Unexpectedly, retinas from high-fat-fed mice manifested striking induction of stress kinase and neural inflammasome activation at 3 months, before the development of systemic glucose intolerance, electroretinographic defects, or microvascular disease. These results suggest that retinal disease in the diabetic milieu may progress through inflammatory and neuroretinal stages long before the development of vascular lesions representing the classic hallmark of diabetic retinopathy, establishing a model for assessing novel interventions to treat eye disease.

YPSA-31

Regulation of hepatic cholesterol metabolism by LeXis, a lipid-responsive non-coding RNA

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The liver X receptors (LXRs) are key transcriptional regulators of cellular and systemic cholesterol homeostasis. On the other hand, the sterol regulatory element-binding proteins (SREBPs) control cholesterol homeostasis in mammalian cells by inducing the expression of genes involved in cholesterol and fatty acid biosynthesis as well cellular uptake. The molecular mechanisms that integrate LXRs with other regulatory pathways are incompletely understood. Here we show that ligand activation of LXRs in liver not only promotes cholesterol efflux, but also simultaneously inhibits cholesterol biosynthesis. We further identify the novel long non-coding RNA *LeXis* as an unexpected mediator of this effect. Hepatic*LeXis* expression is robustly induced in response to western diet feeding or pharmacologic LXR activation. Raising or lowering the levels of *LeXis* in liver affects the expression of cholesterol biosynthetic genes, and the levels of cholesterol in the liver and plasma. *LeXis* interacts with and gates the DNA binding of the heterogeneous ribonucleoprotein Raly, a transcriptional coactivator that is required for the maximal expression of cholesterologenic genes. Targeted genetic disruption of *LeXis* in mice markedly alters transcription of cholesterol biosynthetic genes and hepatic cholesterol homeostasis. These studies outline a regulatory role for a non-coding RNA in lipid metabolism and advance our understanding of the mechanisms orchestrating sterol homeostasis.

YPSA Poster Abstracts

YPSA-32

Effects of vertical sleeve gastrectomy on HDL function in adolescents with severe obesity

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Severe obesity is a major problem among US adolescents, and traditional weight loss strategies, including lifestyle intervention, have had limited efficacy. Bariatric surgery has grown in popularity because it results in rapid weight loss while improving cardiovascular disease risk factors, including raising high-density lipoprotein cholesterol (HDL-C). Whether this rise in HDL-C translates into improved HDL cardioprotective function is not clear, particularly in adolescents. We studied the impact of weight loss surgery (vertical sleeve gastrectomy) in adolescents with severe obesity on HDL function pre- and one year post-operatively.

Adolescents underwent laparoscopic vertical sleeve gastrectomy between 2008 and 2011. HDL function (cholesterol efflux, HDL oxidation, and HDL inflammatory index) was measured pre- and post-surgery on stored serum (-80°C) after apolipoprotein B depletion with polyethylene glycol. Only males were studied in this pilot study to minimize the known effects of sex and the menstrual cycle on lipoproteins. Changes pre and post-surgery were evaluated using paired t-tests.

Participants (n=10, 90% Caucasian) were a mean±SD age of 17.4±1.6 years at baseline and 18.4 ±1.5 years at follow-up. After vertical sleeve gastrectomy, BMI was 32% lower than baseline ($p<0.01$). All lipid measurements improved, and HDL-C increased by 23%. HDL function measured by cholesterol efflux, HDL oxidation potential, and HDL inflammatory index significantly improved post-vertical sleeve gastrectomy significantly compared to baseline, all $p<0.01$.

We conclude that vertical sleeve gastrectomy results in a significant improvement in HDL cholesterol levels and HDL function in adolescents with severe obesity. Whether these changes result in long term improvement in cardiovascular health remains to be determined.

YPSA-33

An extracellular RNA signature of insulin resistance

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Insulin resistance remains a hallmark of metabolic dysfunction in human obesity. Animal models suggest exquisite regulation of circulating extracellular RNAs (ex-RNAs) in the development/resolution of obesity and in metabolic crosstalk between various organs involved in adipocyte dysfunction, though absence of validation in large at-risk populations has limited clinical translation. In this study, we measured a panel of commonly expressed extracellular RNAs in 2,763 individuals (median age 66, body mass index BMI 27.7 kg/m^2) in the 8th Examination of the Framingham Heart Study (FHS) Offspring cohort. Using linear models adjusted for age and sex to identify detectable ex-RNAs associated with insulin resistance (IR; defined as homeostatic model of insulin resistance and insulin alone), we found 18 ex-RNAs associated with IR, many of which remained after adjustment for diabetes and BMI and some of which had been previously implicated in basic and human studies of IR. Of these ex-RNAs, two ex-RNAs (miR-122 and miR-192) were consistently associated with imaging indices of central adiposity, including visceral fat quantity and fat attenuation by computed tomography (imaging measures of dysfunctional adiposity), hepatic fat, and visceral to subcutaneous fat ratio, but not with subcutaneous fat. In a separate cohort of 90 obese/overweight children (mean age 15.5±4.8 years, 60% female), miR-122 was again associated with IR, independent of age, sex, and BMI. Using untargeted metabolomics and principal component analysis (PCA), we demonstrated that miR-122 was significantly associated with IR after adjustment for age, sex, BMI, and different metabolites. Through review of known targets of miR-122 and bioinformatics interrelating ex-RNA-mRNA targets-downstream metabolites, we found that miR-122 regulates key metabolic pathways, including a potential roles in lipid and branched chain amino acid biology. These results provide large-scale evidence relating ex-RNAs to IR in humans and inform future translational and basic investigations linking metabolism, circulating ex-RNAs, and obesity to IR.

YPSA Poster Abstracts

YPSA-34

High-fat diet-induced remodeling of PPARγ binding sites in mouse visceral white adipose tissue

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The nuclear receptor PPARγ is the master regulator of fat cell development and implicated in type 2 diabetes. High-fat diet (HFD)-induced obesity in mice is a common model of metabolic syndrome, in which white adipose tissue (WAT) becomes infiltrated with macrophages. PPARγ is also expressed in macrophages, where we have previously reported a set of unique genomic binding sites not present in cultured adipocytes. Here we describe genome-wide binding regions (cistromes) for PPARγ in WAT from male C57BL/6 mice fed a control low-fat diet versus HFD (10% or 60% calories from fat) for 4 or 12 weeks, which resulted in marked remodeling of the PPARγ cistrome in epididymal WAT (eWAT). Remarkably, PPARγ sites gained in eWAT upon HFD were highly enriched for macrophage-selective binding sites. These were lost in eWAT of mice with macrophage-specific PPARγ deletion, confirming their macrophage-specificity and indicating detectable PPARγ genomic binding events in WAT-resident macrophages. These changes in PPARγ occupancy correlated with nearby mRNA expression, as genes involved in macrophage and inflammatory pathways are induced in eWAT from HFD-fed mice. Conversely, loss of PPARγ binding in eWAT upon HFD was observed near adipocyte-selective binding sites, and the HFD-repressed genes are involved in adipocyte and metabolic pathways. Similar dynamic patterns were observed at the epigenome, notably in histone acetylation. This integrative genomic analysis revealed that HFD-regulated changes in adipose tissue PPARγ cistromes begin early, prior to marked weight gain, inflammation, and insulin resistance, differentially impacting the function of adipocytes and adipose tissue macrophages.

YPSA-35

Tubule-specific β-catenin signaling contributes to glomerular injuries

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Wnt/β-catenin is an evolutionarily conserved signaling pathway important in kidney development. Although quiescent in adults, this pathway is re-activated in disease and contributes to pathology. Previous evidence has shown that activation of glomerular β-catenin expression causes glomerular injury and proteinuria, but whether tubule-specific β-catenin activation contributes to these injuries is unknown.

To examine this, we crossed β-catenin floxed mice with mice expressing Cre under the tubule-specific Ksp promoter, resulting in tubule-specific knockouts for β-catenin (Ksp-β-catenin). To induce proteinuria, we exposed these mice to angiotensin II (1.5 mg/kg/day), or to adriamycin (14 mg/kg). Urinary albumin-to-creatinine ratios were determined. Nephrin and Wilms tumor 1 (WT1) levels were quantitated, and electron microscopy was performed on mouse glomeruli. Fibrosis levels were determined with histology and fibronectin levels, and mRNA levels of soluble injury markers were also assessed.

All mice developed albuminuria after angiotensin II exposure, but Ksp-β-catenin mice were significantly protected from injury. We obtained similar results with the adriamycin model, demonstrating the generalizability of this effect. The albuminuria was not accompanied by increases in low-molecular-weight proteins, suggesting that excretion originated from glomeruli rather than the tubules. In support of this, we found that angiotensin-treated control littermates expressed less WT1 and nephrin and exhibited worsened podocyte foot process effacement compared with Ksp-β-catenin mice, suggesting glomerular and podocyte injury. Interstitial fibrosis was also higher in the wild-type mice. These changes were associated with increases in pathologic soluble mediators that may mediate these injuries via tubular-glomerular crosstalk.

Tubule-specific β-catenin activation plays a key role in the development of glomerular injuries, primarily through increases in podocyte injury via soluble mediators. Our studies suggest a novel β-catenin-mediated tubular-glomerular crosstalk in the pathogenesis of proteinuric kidney diseases.

YPSA Poster Abstracts

YPSA-36

Daily mean temperature and clinical kidney stone presentation in five metropolitan areas in the United States: a time-series analysis

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Observed geographic and seasonal differences in nephrolithiasis rates implicate temperature in the causal pathway of nephrolithiasis, but the precise relationship between temperature and kidney stone presentation is unknown. Our objective was to estimate the temperature dependence of kidney stone presentation.

Using a time-series design and distributed lag nonlinear models, we estimated the relative risk (RR) of kidney stone presentation associated with mean daily temperatures, including cumulative RR for a 20-day period, and RR for individual daily lags through 20 days. We used data from the MarketScan Commercial Claims database for 60,433 patients who sought evaluation or treatment of nephrolithiasis from 2005 to 2011 in Atlanta, Chicago, Dallas, Los Angeles, and Philadelphia.

Associations between mean daily temperature and kidney stone presentation were not monotonic, and there was variation in the exposure–response curve shapes and the strength of associations at different temperatures. However, in all cities except Los Angeles, RRs increased for temperatures above the reference value of 10°C. The cumulative RR for a daily mean temperature of 30°C versus 10°C was 1.38 in Atlanta (95% confidence interval [CI]: 1.07, 1.79), 1.37 in Chicago (95% CI: 1.07, 1.76), 1.36 in Dallas (95% CI: 1.10, 1.69), 1.11 in Los Angeles (95% CI: 0.73, 1.68), and 1.47 in Philadelphia (95% CI: 1.00, 2.17). Kidney stone presentations were also positively associated with temperatures <2°C in Atlanta, and <10°C in Chicago and Philadelphia. In four cities, the strongest association between kidney stone presentation and a daily mean temperature of 30°C versus 10°C was estimated for lags of ≤3 days.

Generally, kidney stone presentations increased with hot and cold daily temperatures, with the strongest associations estimated for lags of a few days. These findings support an adverse effect of temperature extremes on nephrolithiasis risk.

YPSA-37

Pancreatic circulating tumor cell heterogeneity identified by single-cell RNA sequencing

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Circulating tumor cells (CTCs) are thought to be enriched for cells with metastatic potential and their characterization offers insight into the biological underpinnings of the distal spread of cancer. We have employed a novel microfluidic isolation device to capture high numbers of CTCs without antigen bias called the CTC-iChip. This device achieves high efficiency negative depletion of normal blood cells providing an enriched population of CTCs in solution that are not biased by a particular extracellular epitope and are captured without any antibody interactions that could affect expression profiles or cell viability. We have utilized this platform to fully characterize the heterogeneity of CTCs using single-cell RNA sequencing. In the genetically engineered pancreatic cancer mouse model, we were able to demonstrate that 55% of individual CTCs did not have sufficient RNA for sequencing indicating that they were not viable. Of the 45% of viable CTCs, we were able to identify 3 distinct classes of CTCs: (1) classical CTCs (CTC-c) defined by established keratin expression, biphenotypic EMT markers, and enrichment of stem cell markers; (2) platelet adhered CTCs (CTC-plt); and (3) proliferative CTCs (CTC-pro). Notably, CTC-c was the most prevalent CTC class and was found to have extremely high expression of extracellular matrix proteins in both mouse and human, suggesting that the "seeds" of metastasis produce their own "soil." The functional differences between these subtypes remains to be determined, but this work has revealed the true heterogeneous nature of CTCs in the metastatic cascade. The transcriptional signatures of these defined CTC subpopulations are being applied to blood samples from patients with pancreatic cancers to determine their utility in increasing the sensitivity and specificity of CTCs as biomarkers for early detection and response to therapy.

YPSA Poster Abstracts

YPSA-38

Radiation and dual immune checkpoint blockade overcome tumor resistance and distinctly improve immunity

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Pre-clinical and clinical data indicate that radiation (RT) may augment responses to immune checkpoint inhibition. We therefore evaluated this combination for metastatic melanoma using parallel studies in mice and humans. In a phase I clinical trial with 19 patients with multiple melanoma metastases, a single index lesion was irradiated with hypofractionated RT followed by four cycles of the anti-CTLA4 antibody ipilimumab. We reproduced this therapy in mice using the melanoma cell line B16-F10. For this, each flank of C57BL/6 mice was implanted with tumors to model multiple metastases. Mice received anti-CTLA4 (on days 5, 8, and 11), irradiation of one tumor using an image-guided micro-irradiator (20 Gy x 1 on day 8), or both treatments. Mechanistic studies were performed on material obtained from patients and mice at baseline and thereafter. Overall, treatment in the phase I study was well tolerated and toxicity was similar to that reported for anti-CTLA4. Major tumor regressions were observed in a subset of patients with metastatic melanoma treated with anti-CTLA4 + RT. In mice, although combined treatment enhanced the CD8 T cell:Treg ratio and improved responses, resistance was common. Genome-wide analyses revealed that resistant tumors have increased PD-L1, interferon-stimulated genes, and exhausted T cells that suppress the CD8/Treg ratio. Patients and mice with high PD-L1 tumors that were treated with RT + anti-CTLA4 poorly reinvigorated exhausted T cells, did not respond, and rapidly progressed. In mice, adding anti-PD-L1/PD-1 to RT + anti-CTLA4 reinvigorated exhausted T cells, leading to complete responses. Our clinical and pre-clinical data suggest that the combination of RT with CTLA4 and PD-1 checkpoint blockade is a rational, non-redundant approach to overcoming tumor resistance and improving immunity in metastatic melanoma and potentially other tumors (in data not in this abstract).

YPSA-39

Identifying resistance mechanisms in ER+ metastatic breast cancer by translational genomics

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In spite of great progress in the treatment of estrogen receptor-positive (ER^+) metastatic breast cancer (MBC) using ER-directed agents, patients invariably develop resistance to these therapies. These resistant tumors remain the most common cause of breast cancer death, yet mechanisms by which this resistance develops are poorly understood. To identify these resistance mechanisms, we performed whole exome sequencing (WES) and transcriptome sequencing (RNASeq) on metastatic tumor biopsies from 100 patients with ER^+ breast cancer who had developed resistance to one or more ER-directed therapies. In a subset of these patients, we also performed WES and RNA-seq on treatment-naïve primary tumors for comparison to the resistant specimens. WES data were analyzed for base substitutions, small insertions/deletions, and copy number alterations. RNA-seq data were analyzed for fusions, chimeric read-through transcripts, point mutations, and instances of alternative splicing. RNA-seq data was also used to determine gene expression levels. Putative resistance mechanisms identified in the resistant samples could generally be classified into three main categories: (i) *secondary alterations in the target,* including ESR1 mutations and amplifications, (ii) *bypass or compensatory alterations*, including ERBB2 activating mutations and alterations in cell-cycle proteins such as Rb, and (iii) *ER indifference,* including the loss of the estrogen receptor. The specific resistance mechanisms identified suggest potential therapeutic approaches for overcoming or preventing resistance. Overall, these data illuminate the genomic landscape of resistant ER^+ MBC and sheds light on how ER^+ breast cancers develop resistance to targeted therapies, thereby aiding the identification of new targets and the development of novel therapeutic strategies for patients with resistant breast cancer.

YPSA Poster Abstracts

YPSA-40

Incomplete sodium channel inactivation causes spontaneous atrial fibrillation *in vivo* and ex vivo in a novel transgenic mouse

***Elaine Y. Wan*, Jeffrey Abrams, Richard L. Weinberg, Alexander Katchman, Sergey I. Zakharov, Lin Yang, Joseph Bayne, John Morrow, Hasan Garan, Steven O. Marx**

Columbia University, New York

The underlying mechanisms for the initiation and perpetuation of atrial fibrillation (AF) are unclear. Both gain-of-function and loss-of-function mutations in SCN5A are associated with an increased incidence of AF. Furthermore, acquired defects in Na^+ channel inactivation have been described in patients with chronic AF. In a transgenic mouse line expressing human NaV1.5 with a mutation in the local anesthetic binding site (F1759A), spontaneous and prolonged episodes of AF were observed.

Whole cell patch and calcium imaging were performed on cardiomyocytes of control and F1759A-Tg mice. Echocardiography, histology, transmission electron microscopy (TEM), and optical mapping were performed *in vivo* and ex vivo.

Atrial and ventricular cardiomyocytes isolated from the F1759A-Tg mice had increased persistent Na^+ current. Increased persistent Na^+ current in the atria and ventricles was sufficient to cause mildly reduced cardiac contractility, increased atrial size, increased atrial fibrosis, myofibril disarray, and mitochondrial necrosis. Spontaneous and prolonged episodes of paroxysmal AF were observed during optical mapping in eight Tg mice. AF was not observed in eight non-Tg mice. The APD80 was prolonged, 4-fold in the left atrium and 2.5-fold in the right atrium of Tg mice compared to controls. There was also increased APD dispersion in Tg atria. Optical mapping showed phase singularities and wave reentry emanated from areas of high spatio-temporal gradients. There was a direct correlation between dominant frequency and regularity indices. Based upon our hypothesis that incomplete Na^+ channel inactivation caused Ca^{2+} overload thereby promoting arrhythmogenesis, Tg mice were administered a single IP injection of the inhibitor of Na^+-Ca^{2+} exchange, SEA0400. AF burden was reduced by 59% (n=5 mice).

Incomplete Na^+ channel inactivation is sufficient to initiate the structural and arrhythmogenic substrates required to initiate and perpetuate AF in mice. These mice offer a new approach to understand and treat atrial fibrillation.

APSA Congratulates the AAP on its 130th Anniversary

On behalf of Moshe Levi – Chair of the Board of Directors, and Daniel DelloStritto – President, the American Physician Scientists Association (APSA) would like to applaud the Association of American Physicians (AAP) on their 130th anniversary. Those 130 years have witnessed astonishing advances in science and our understanding of human health and disease, and many of these advances have arisen directly from the work of AAP members.

Looking through the history of the AAP, one can see many themes that emerge, subside and reemerge in time. One which has always been held dear is the importance of science to advancement of medicine and human health and of the need to support physician-scientists in training to advance science in medicine. In one of the very first presidential addresses given in the 20th century, then-President of the AAP William H. Welch declared that hospitals of their time should "enable promising young men to do scientific work, to acquire thorough clinical experience, and to begin to establish their reputations by contributions to their special departments of knowledge." (Transactions of the Association of the American Physicians; 1901) This clearly shows the deep investment the Association has made since its founding to foster the next generation of young physician-scientists.

Indeed, this commitment to mentoring the next generation has never wavered, and APSA has been the beneficiary of the AAP's generous support since its founding 12 years ago. APSA is grateful to the AAP for graciously welcoming the youngest generation of physician-scientists trainees to walk amongst them and to learn from their examples and their advice. Joining AAP members at the Joint Meeting is, for APSA members, to witness the "glitterati of American academic medicine— the mythical heroes [we] had read and heard about" (2001: An AAP Odyssey, Robert J. Lewkowitz's AAP Presidential Address).

The example set by the AAP Is a constant source of inspiration for APSA members. The achievements of the AAP in science, in medicine, and in leadership of both fields embody the aspirations of APSA trainees and serve as a living reminder of the heights to which our members themselves someday hope to climb.

APSA congratulates the AAP on 130 years leading advances in human health through the science of medicine. May the next 130 be even grander.

Sincerely,

Moshe Levi, Chair of the Board

Daniel DelloStritto, President

HHMI Medical Fellows Presenters

Leonel Ampie (Poster: HHMI-1)
School: Georgetown University School of Medicine; Fellowship: Northwestern University, The Feinberg School of Medicine

Sriram Anbil (Poster: HHMI-2)
School: The University of Texas School of Medicine at San Antonio; Fellowship: Harvard Medical School

Nicholas Andresen (Poster: HHMI-3)
School and Fellowship institution: University of Iowa Roy J. and Lucille A. Carver College of Medicine

Courtney Baker (Poster: HHMI-4)
School and Fellowship institution: Vanderbilt University School of Medicine

Leandra Barnes (Poster: HHMI-5)
School and Fellowship institution: Stanford University School of Medicine

Jacob Basham (Poster: HHMI-6)
School: University of Tennessee Health Science Center College of Medicine; Fellowship: St. Jude Children's Research Hospital

Joseph Bayne (Poster: HHMI-7)
School and Fellowship institution: Columbia University College of Physicians and Surgeons

Tedi Begaj (Poster: HHMI-FFB-8)
School: University of Massachusetts Medical School; Fellowship: Harvard Medical School

Caitlin Bell (Poster: HHMI-9)
School: Vanderbilt University School of Medicine; Fellowship: Harvard Medical School

Harjus Birk (Poster: HHMI-10)
School and Fellowship institution: University of California, San Francisco, School of Medicine

John Bui (Poster: HHMI-11)
School and Fellowship institution: University of Pittsburgh School of Medicine

Elizabeth Buss (Poster: HHMI-12)
School: Sidney Kimmel Medical College at Thomas Jefferson University; Fellowship: Harvard Medical School

Rima Chakrabarti (Poster: HHMI-13)
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Allison Dobry (Poster: HHMI-20)
School and Fellowship institution: Harvard Medical School

James Drake (Poster: HHMI-21)
School and Fellowship institution: University of Michigan Medical School

Kow Essuman (Poster: HHMI-22)
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Allison Hamilos (Poster: HHMI-28)
School and Fellowship institution: Harvard Medical School

David Huang (Poster: HHMI-29)
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John Huizar (Poster: HHMI-30)
School and Fellowship institution: University of California, San Francisco, School of Medicine

Soyun Hwang (Poster: HHMI-OREF-31)
School and Fellowship institution: Mayo Medical School

Sally Ingham (Poster: HHMI-32)
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Vishesh Khanna (Poster: HHMI-36)
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Arin Kim (Poster: HHMI-37)
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Jonathan Massie (Poster: HHMI-45)
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Warren Woodrich Pettine (Poster: HHMI-58)
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Jose Porras (Poster: HHMI-59)
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Briana Prager (Poster: HHMI-60)
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Jessica Ruiz (Poster: HHMI-63)
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Alireza Samiei (Poster: HHMI-PDF-64)
School and Fellowship institution: Harvard Medical School

Zahra Sayyid (Poster: HHMI-65)
School and Fellowship institution: Stanford University School of Medicine

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Montgomery Simms (Poster: HHMI-67)
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Sumi Sinha (Poster: HHMI-68)
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Nathanael Smith (Poster: HHMI-69)
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Evelyn Song (Poster: HHMI-71)
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Kun Wei Song (Poster: HHMI-CURE-72)
School and Fellowship institution: Duke University School of Medicine

Jeffrey SoRelle (Poster: HHMI-70)
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Tin-Yun Tang (Poster: HHMI-73)
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Jack Turban (Poster: HHMI-75)
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School and Fellowship institution: Washington University in St. Louis School of Medicine

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School and Fellowship institution: Yale School of Medicine

(Julia) Erin Wiedmeier (Poster: HHMI-80)
School and Fellowship institution: Oregon Health and Science University School of Medicine

Noah Williford (Poster: HHMI-81)
School and Fellowship institution: University of Iowa Roy J. and Lucille A. Carver College of Medicine

Patrice Witschen (Poster: HHMI-BWF-82)
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Wonhee Woo (Poster: HHMI-83)
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Amy Z. Xu (Poster: HHMI-84)
School and Fellowship institution: Washington University in St. Louis School of Medicine

Daniel Yang (Poster: HHMI-85)
School: Yale School of Medicine; Fellowship: Harvard Medical School

Xiao Zhu (Poster: HHMI-86)
School and Fellowship institution: University of Pittsburgh School of Medicine

Howard Hughes Medical Institute Medical Fellows Poster Abstracts

HHMI-1

Galectin-3 mediates glioma-induced immunosuppression through expansion of regulatory T cells

Leonel Ampie,[1,2] Winward Choy,[1] Jonathan B. Lamano,[1] Orin Bloch[1]

[1]Department of Neurological Surgery, Northwestern University, Feinberg School of Medicine; [2]Howard Hughes Medical Institute

Galectin-3, a carbohydrate-binding lectin, has been demonstrated to correlate with tumor grade within brain gliomas. Glioblastoma (GBM), a grade IV glioma, represents the most common primary brain tumor in adults which portends a poor prognosis of only 16 months median survival. GBM also promotes the expansion of circulating and intratumoral regulatory T-cells (Tregs) which may hamper a proinflammatory response against the tumor. We hypothesize that Galectin-3 is secreted by GBM and is a key player in the promotion of Treg expansion.

Conditioned media from primary (n=8) and established (n=1) glioma cell lines were analyzed for Galectin-3 secretion by ELISA. Cell lysates were analyzed for intracellular Galectin-3 via immunoblotting and localization was confirmed by immunohistochemistry of GBM tissue. Healthy control lymphocytes were activated with anti-CD3/CD28 and recombinant Galectin-3, with assessment of lymphocyte phenotype by flow cytometry on day 3 of incubation. Treg fraction ($CD4^+/CD25^+/FoxP3^+$) and cell surface immune-checkpoint induction were analyzed.

Four of eight primary patient cell lines secreted varying levels of Galectin-3 ranging from 0.2-1.7ng/mL. Results were verified via immunoblotting which correlated with secretion by ELISA. Stimulation of healthy lymphocytes with recombinant Galectin-3 resulted in increased Treg expansion compared to unstimulated controls (30% vs. 14.2%, p=0.0002). Analysis of immune checkpoints on $CD4^+$ cells revealed an increase in LAG-3 expression with Galectin-3 stimulation compared to unstimulated controls (30% vs. 22.8%, p=0.0014).

For the first time, we demonstrate that Galectin-3 can induce Treg expansion and LAG-3 expression on $CD4^+$ cells. We also demonstrate that Galectin-3 is secreted by a subset of GBM tumors, contributing to the immunosuppressive environment observed in patients. Our data suggests that Galectin-3 may be a novel target for immunomodulation in GBM.

HHMI-2

Biomodulation of metabolic and signaling pathways to enhance photodynamic therapy efficacy for pancreatic adenocarcinoma and oral squamous cell carcinoma

Sriram Anbil,[1,2,3] Imran Rizvi,[2,4] Mans Broekgaarden[2], Yan Baglo,[2] Edward Maytin,[5] Tayyaba Hasan[2]

[1]The University of Texas School of Medicine at San Antonio; [2]Harvard Medical School, Massachusetts General Hospital, Wellman Center for Photomedicine; [3]Howard Hughes Medical Institute; [4]Brigham and Women's Hospital; [5]Cleveland Clinic Lerner College of Medicine

Vitamin based biomodulation of metabolic and paracrine-signaling pathways represents a promising approach to overcome tumor heterogeneity and treatment escape. Among these approaches includes biomodulation of the heme pathway with calcitriol to increase accumulation of protoporphryin IX (PpIX), the photosensitizer responsible for the cytotoxic effect of aminolevulinic acid (ALA)-based photodynamic therapy (PDT). Because cancer associated desmoplasia has been implicated in treatment resistance in several contexts including pancreatic adenocarcinoma (PDA) and oral squamous cell carcinoma (OCa), an opportunity exists to increase the susceptibility of these tumors to PDT through stromal rehabilitation while also improving the therapeutic index via heme pathway biomodulation. We investigate the ability of the vitamin hormones calcipotriol or retinoic acid to serve as dual-purpose biomodulatory and stromal reprogramming agents to enhance PDT efficacy in context of OCa and PDA. OCa is highly prevalent in low resource settings where few feasible modalities exist for early detection and treatment. In contrast, PDA is frequently detected at late stage and characterized by a unique milieu comprising activated mesenchymal cells that facilitate tumor progression. Our approach has significance in both contexts: it may improve the theranostic potential of low-cost image guided PDT for OCa, and may enable synergy between PDT and other chemo/biologic therapies for PDA via modulation of tumorigenic signaling pathways. Using *in-vitro* and *in-vivo* approaches, we demonstrate the ability of vitamin hormones to improve the selectivity and homogeneity of PpIX accumulation in OCa and PDA. In 2D and 3D cultures, we demonstrate the ability of calcipotriol and retinoic-acid to induce quiescence in activated fibroblast lines, and assess the impacts of this "reprogramming" in PDA lines. A combined biomodulatory and reprogramming approach is shown to improve PDT efficacy in OCa and PDA lines.

Howard Hughes Medical Institute Medical Fellows Poster Abstracts

HHMI-3

Development of *Ariolimax* as a model for studying mucus formation

Nicholas S. Andresen, **Lin Lu, Lynda S. Ostedgaard, Thomas O. Moninger, Michael J. Welsh**

Howard Hughes Medical Institute and Departments of Internal Medicine and Molecular Physiology and Biophysics and Central Microscopy Research Facility, Roy J. and Lucille A. Carver College of Medicine, University of Iowa

Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) anion channel. The primary cause of CF morbidity and mortality is airway infection, inflammation, mucus accumulation, and airway obstruction. Studies of a porcine model of CF have shown that prior to the onset of infection and inflammation, mucus with abnormal biophysical properties impairs mucociliary transport. Abnormal pH and/or Ca^{2+} concentrations in CF are hypothesized to alter airway mucus. The goal of this work was to develop a model to understand how pH and Ca^{2+} levels affect mucus properties at the time mucus is released from its packed state in membrane-bound vesicles. We used the terrestrial slug *Ariolimax*, which secretes 10 µm membrane-enclosed granules that are packed with mucus. We developed methods to collect and store mucus granules. When we ruptured the granule membrane, mucus volume increased ~200-fold. Scanning election microscopy revealed rope-like mucus strands reminiscent of those observed with porcine airway mucus. Fluorescent lectins and histochemical stains that are used to stain porcine airway mucus also labeled *Ariolimax* mucus. Mucus granules expanded to the same volume at pH 5 and at pH 9. However, when we tested the cohesiveness of mucus by measuring its ability to enter capillary tubes via capillary action, we found that mucus expanded at pH 9 traveled further than mucus expanded at pH 5. Adding 5 or 10 mM Ca^{2+} reduced mucus volume and decreased mucus movement in capillaries. Thus, pH and Ca^{2+} concentration are key variables that affect *Ariolimax* mucus. These findings provide a highly tractable model for future studies focused on the abnormalities of CF mucus.

HHMI-4

Osteopontin deficiency contributes to heterotopic ossification

Courtney Baker, **[1] Jonathan Schoenecker[2]**

[1]Vanderbilt University School of Medicine, Howard Hughes Medical Institute; Research Fellowship; [2]Vanderbilt University Department of Orthopaedics, Pediatrics, Pharmacology, Pathology

Heterotopic Ossification (HO) is the aberrant formation of bone within soft tissues and a major source of morbidity and expenditure in both the military and civilian populations following burn, blast, and neurologic injuries. It was recently published that plasminogen is essential for preventing HO, but plasmin has no known direct anti-mineralization properties. Further experiments demonstrated plasmin's mechanism to prevent HO is independent of fibrinolysis. Osteopontin (OPN) is produced by macrophages and injured muscle, has significant anti-mineralization properties, and is a known plasmin substrate. We hypothesized that the injury provoked anti-mineralization activity of plasmin is interdependent with OPN activity. Selective muscle injury of OPN heterozygous and homozygous null mice demonstrated a variable incidence of HO that regressed with time via serial radiographs. OPN homozygous null mice demonstrated more severe HO than heterozygous mice and both were significantly greater than controls. Female mice demonstrated more severe HO than matched males, which is suggestive of sexual dimorphism in this injury repair model. Selective pharmacologic reduction in OPN levels of wild type mice also demonstrated a dose response of HO after muscle injury. Raising plasmin activity in OPN heterozygous mice prevented HO formation compared to controls. This suggests that combined selective muscle injury and OPN deficiency is a novel model of HO demonstrating variable incidence, regression, and sexual dimorphism and that increasing plasmin activity can prevent this phenotype.

HHMI-5

Transplantation of M2 macrophages improves cutaneous wound repair *in vivo*

Leandra A. Barnes, **[1,2] Michael S. Hu,[1,3] Tripp Leavitt,[1] Clement D. Marshall,[1] Alexander T.M. Cheung,[1] Michael T. Longaker[1]**

[1]Stanford University; [2]Howard Hughes Medical Institute; [3]University of Hawai'i

Skin defects pose a significant reconstructive challenge, so it is necessary to develop novel approaches in regenerative medicine to improve tissue repair. Studies have shown the macrophage is the only inflammatory cell whose deletion slows healing, indicating its critical role in skin wound repair. Macrophages can be polarized to two phenotypes that have different functions: M1 phenotype macrophages are pro-inflammatory, while M2 phenotype macrophages are pro-regenerative. Using an established humanized excisional wound model in mice, we demonstrate the ability of M2 polarized macrophages to promote skin regeneration *in vivo*. Monocytes were isolated from the bone marrow of mice expressing green fluorescent protein constitutively in the cytoplasm of all cells, differentiated into macrophages *in vitro* with macrophage colony-stimulating factor, seeded onto biomimetic hydrogel scaffolds containing recombinant interleukin-10 (IL-10) for *in vivo* polarization to the M2 phenotype, and transplanted onto splinted excisional wounds. Gross wound progression and size was assessed with digital photographs. Wound tissue was harvested for histologic, gene expression, and cellular analyses. Gene expression

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analysis confirmed that macrophages seeded onto the scaffold containing IL-10 were polarized to the M2 phenotype *in vivo* within 48 hours. Polarizing macrophages to the M2 phenotype on an IL-10 scaffold *in vivo* minimizes time in cell culture and proves to be optimal for directed cell delivery. Our results demonstrate that supraphysiologic levels of M2-polarized macrophages significantly accelerate wound healing without adverse effects on scar size and quality.

HHMI-6

Opposing effects of programmed death-1/ programmed death ligand-1 engagement and IFN-γ in the treatment of AML with anti-CD33 chimeric antigen receptor-modified T cells

***Jacob H. Basham,*[1,2] Wenting Zheng,[2] Terrence L. Geiger[2]**

[1]Howard Hughes Medical Institute; [2]St. Jude Children's Research Hospital

Chimeric antigen receptor-modified T lymphocytes (CART cells) have shown benefit as an adjuvant immunotherapy in the treatment of B cell malignancies. This success of re-targeted T cells has not been extended to other hematologic malignancies. We have developed an immunotherapeutic approach to treat acute myeloid leukemia (AML) using CART cells re-directed against the myeloid-specific antigen CD33 (CART-33). CART-33 cells are potent and specific in eliminating AML cells *in vitro* and *in vivo*. Despite this, CART-33 T cells have shown poor *in vivo* expansion in NOD-SCID IL2rγ (-/-) (NSG) AML xenograft models. To address the reason for this, we assessed the impact of AML-expressed programmed death ligand 1 (PD-L1) on CART-33 cell activity. PD-L1 inhibits T cell functions upon binding PD-1, which is upregulated with T cell activation. Interferon-gamma (IFN-γ), a primary effector cytokine secreted by $CD8^+$ effector T cells, is a potent inducer of PD-L1 on AML blasts. Using AML cell lines U937, Oci-AML3, CMK, and MV4-11 we show that both IFN-γ and activated CART-33 supernatant can induce up-regulation of PD-L1 on AML. Upregulation is pre-dominantly though not exclusively dependent upon IFN-γ production. The kinetics and induction of a corresponding PD-L family member, PD-L2 is distinct from that of PD-L1. Although PD-L1 is well documented to suppress T cell function, induction of PD-L1 had no effect on the cytolytic activity of CART-33 cells against AML in short term (<48 h) cultures. Paradoxically, 24 hr pre-treatment of AML with either IFN-γ or CART-33 supernatant increased AML susceptibility to killing by CART-33 cells despite elevated expression of PD-L1 by AML. Our results highlight the regulatory complexity of AML cytolysis by re-targeted T lymphocytes, and argue that that tumor-expressed PD-L1 impacts the sustainability but not short-term killing activity of adoptively transferred CAR T cells in the treatment of AML.

HHMI-7

Persistent sodium current and its role in cardiac arrhythmias and structural remodeling of the atria and ventricles

Joseph Bayne,[1,2] Jeffrey Abrams,[2] Steven O. Marx[2]

[1]Howard Hughes Medical Institute; [2]Division of Cardiology, Department of Medicine, College of Physicians and Surgeons, Columbia University

Nav1.5, encoded by the *SCN5A* gene, initiates the cardiac action potential and the loading of cardiomyocytes with sodium ions. Increased sodium current through this channel by incomplete inactivation has been associated with long QT syndrome, atrial fibrillation and dilated cardiomyopathy in humans. To explore the molecular mechanisms underlying these phenotypes, we created doxycycline-inducible transgenic mice with a mutation (F1759A) of the local anesthetic binding site in human *SCN5A*. The F1759A mutation causes incomplete inactivation of the sodium channel, assessed by patch clamp. The F1759A transgenic mice developed spontaneous and prolonged episodes of atrial fibrillation, an unusual phenotype in mice. The arrhythmia occurred in the setting of left atrial enlargement and mild fibrosis, and mild left ventricular enlargement and dysfunction, phenocopying the findings observed in humans with various mutations of *SCN5A*. To explain the causality of the *SCN5A* mutation towards these structural changes, deep transcriptome sequencing (RNA sequencing) was performed after inducing F1759A Nav1.5 expression for 7 days. DEseq analysis demonstrated increased expression of 1,000 genes and decreased expression of close to 800 genes in the dox-fed mice. Gene ontology analysis showed that overexpressed genes were associated with inflammation and phosphate transport and that decreased genes were associated with mitochondrial membrane and mitochondrial matrix proteins. These data suggest that increased persistent sodium current is sufficient to cause arrhythmia and cardiac structural changes such as hypertrophy and fibrosis. RNA sequencing analysis suggests that these changes may be secondary to increased expression of genes associated with inflammation and altered expression of genes associated with mitochondria and phosphate transport.

HHMI-FFB-8

AAV-mediated generic gene therapy targeting glucose metabolism in cones of retinitis pigmentosa mouse models

***Tedi Begaj,*[1,3] Yashodhan Chinchore,[2] Connie Cepko[2,3]**

[1]University of Massachusetts Medical School; [2]Harvard Medical School; [3]Howard Hughes Medical Institute and Foundation Fighting Blindness

Progressive, heterogeneous and inherited retinal degeneration collectively known as retinitis pigmentosa (RP) affect around 1.5 million people worldwide, causing severe vision impairment

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and blindness. In many cases, rod cell demise occurs in the first two decades of life followed by a slow decline in visual acuity and central vision attributed to cone cell death. The common feature in cones of various RP models is the indication of metabolic stress after rod cell death. Systemic as well as genetic approaches targeting insulin signaling and mammalian target of rapamycin (mTOR) pathways prolong cone survival in mouse models of RP. Thus gene therapies involving metabolic intervention offer a promising generic approach to prolong cone survival and/or function, regardless of the original rod-specific mutation and with lesser systemic side-effects. We have constructed adeno-associated virus (AAV) vectors encoding genes involved in the central glucose metabolism. The expression from these vectors was confirmed in wild-type as well as diseased retinae. Subretinal injections in 3 different RP models was carried out to assess (1) cone survival, (2) cone function by electroretinography, and (3) visual guided behaviors. The current results will be presented and the implications of these findings will be discussed.

HHMI-9

A novel tissue-specific CRISPR technique elucidates previously unknown tumor suppressors in melanomagenesis

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The study of melanoma mechanisms has been enhanced by the use of a zebrafish model in which human $BRAF^{V600E}$ is driven by the mitf promoter in a p53 mutant background. We generated a vector system that expresses a gRNA and places Cas9 under control of the mitf promoter, leading to melanocyte-specific gene disruption and knockout. Injection of a vector targeting p53 into $BRAF^{V600E+/+}$ $mitfa^{-/-}$ zebrafish embryos leads to melanoma formation in every fish (17 week median onset, n=50), while non-targeting vectors never lead to tumors. As proof of principle, targeting of known melanoma-suppressor pten in $BRAF^{V600E+/+}$ $mitfa^{-/-}$ $p53^{-/-}$ zebrafish leads to significant acceleration of tumor onset (10 week median). We then selected genes found frequently mutated and genomic regions that are deleted at increased rates in human melanomas. For instance, *arid2* encodes a subunit of a chromatin remodeling complex implicated in gene expression and migration of neural crest progenitors, and is one of the most commonly mutated epigenetic factors in melanoma. Inactivation of *arid2* demonstrates earlier tumor onset compared to the control (16 vs. 20 weeks, n>100 $p<10^{-3}$). *Rbfox1*, an alternative splicing factor deleted in 10% of melanomas, accelerates tumor formation compared to the control (17 vs. 20 weeks, n>100 $p<10^{-3}$). We are now using this system to screen entire regions of genes with possible tumor-suppressive function that have frequent biallelic loss. This methodology provides an efficient means for screening genes implicated by robust patient sequencing data, and investigating previously unknown tumor-suppressing roles.

HHMI-10

Identification and characterization of long non-coding RNAs involved in glioblastoma multiforme proliferation

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LncRNAs are a largely uncharacterized class of RNA molecules greater than 200 nucleotides in length that have little protein-coding capacity. Over 50,000 human lncRNAs have been annotated, and many of these transcripts are cell and tissue-specific. In particular, many thousands of lncRNAs are specific to the human brain. Emerging data indicate that lncRNAs play a role in a wide range of brain pathologies including primary tumors such as glioblastoma multiforme (GBM). Characterized by its invasiveness and resistance to treatment, GBM is highly malignant with patients having a median survival 14 months despite surgery, radiation and chemotherapy. Although lncRNAs are particularly cell and disease specific and are attractive as therapeutic targets, very few specific lncRNAs have been identified as potential targets for cancer therapy. To identify and pursue specific lncRNAs as therapeutic targets in human GBM, we conducted a large-scale knockdown screen in the human U87 brain tumor cell line. To assess the role of lncRNAs in GBM cell proliferation, *in vitro* knockdown of lncRNAs were performed and a competitive growth assay using flow cytometry was conducted. GBM cells were grafted into the brains of immunocompromised mice to generate tumors *in vivo*. Bioluminescence imaging was performed weekly in order to visualize and compare tumor growth patterns among control mice and lncRNA knockdown mice. Our knockdown screen identified over 50 lncRNAs involved in GBM proliferation. Knockdown of one specific lncRNA, *LINC00263*, decreased U87 GBM tumor cell propagation in culture by 90% over a three week period. We are currently analyzing the effect of *LINC00263* knockdown on the growth of GBM in a xenograft mouse model. Analysis of such lncRNA targets will lay groundwork for the study of other GBM-associated lncRNAs, having important implications for the development of this new class of RNA transcripts as novel therapeutic targets in GBM.

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HHMI-11

A subset of infectious proviruses persist and expand following activation ex vivo

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The most effective latency reversing agents for HIV-1 are also potent T-cell activators. Recent studies show that virus producing cells can persist and expand *in vivo*. We hypothesized that activation of HIV-infected CD4$^+$ T-cells could lead to clonal expansion of proviruses rather than their elimination. We established an ex vivo cell culture system involving stimulation of patient-derived CD4$^+$ T cells with PMA/ionomycin (day 1-7), followed by rest (day 7-21), and then restimulation (day 21-28) in the presence of raltegravir and efavirenz. Cell-associated HIV-1 DNA (CAD) and virion RNA in the supernatant were quantified by qPCR. Single genome sequencing (SGS) was performed to characterize proviruses and virion RNA. The replication-competence of proviruses in cultured cells was determined by the viral outgrowth assay (VOA) at multiple time points. Experiments were performed with purified CD4$^+$ T-cells from five consecutive donors who had been suppressed on ART for >2 years (median = 13.4 years). In all experiments, HIV-1 RNA levels in supernatant increased following initial stimulation, decreased or remained stable during the rest period, and increased again with restimulation. Cell-associated HIV-1 DNA levels did not show a consistent pattern of change. SGS revealed outcomes of proviral populations including both elimination and expansion. Importantly, a subset of proviruses expanded and produced infectious virus continuously.

HHMI-12

Functional analysis of the retinoblastoma protein in small cell lung cancer

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Small cell lung cancer (SCLC) is a distinct clinical entity within the range of lung cancers. SCLC is the most aggressive form of lung cancer, with a tendency for early dissemination and a median survival of 5-10% at five years. Increasing evidence has pointed to a critical role of the retinoblastoma protein (pRB) in the molecular pathogenesis of small cell lung cancer. Human genomic data has demonstrated that mutation or loss of pRB occurs in essentially all small cell tumors. However, the functional role of pRB loss in small cell lung cancer has not been fully explored. pRB acts as a tumor suppressor in a variety of cancers. Many cancers with wild-type pRB harbor mutations in key regulators of the retinoblastoma pathway such as p16, cdk4, or cyclin D1. Indeed, most cancers are believed to have compromised the pRB tumor suppressor pathway. Interestingly, p16, cdk4, and cyclin D1 mutations are rare in SCLC. This raises the possibility that the tumor suppressive role of pRB in SCLC is not linked to the canonical retinoblastoma pathway. In order to explore the tumor suppressive role of pRB in SCLC, we engineered isogenic RB1$^{-/-}$ SCLC cell lines that inducibly express pRB using a "Tet-On" system. We then performed an unbiased CAS9/CRISPR loss of function screen to identify genes that, when inactivated, conferred a growth advantage in SCLC after induction of pRB. To our surprise we identified known proteins involved in the pRB canonical pathway, such as p16. We also identified a number of other genes that we are interrogating in secondary assays, including assays that utilize SCLC that express wild-type pRB compared to SCLC that express a non-phosphorylatable pRb. This comparison should allow us to place hits upstream, downstream, or parallel of pRB. This information may provide mechanistic insights into tumor suppression by pRB as well as illuminate potential therapeutic opportunities for this disease.

HHMI-13

Adult-onset liver disease and hepatocellular carcinoma in S-adenosylhomocysteine hydrolase deficiency

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The clinical use of exome sequencing has revealed unusual presentations of known inherited diseases. Here we performed whole exome sequencing on a woman of Pakistani descent who was the offspring of a consanguineous mating. She developed elevated aminotransferases at age 23 and was diagnosed with hepatocellular carcinoma at age 29. The patient died with no diagnosis after undergoing liver transplantation at age 32. Sequencing of her exome revealed homozygosity for a missense mutation (R49H) in *AHCY*, the gene encoding S-adenosylhomocysteine (SAH) hydrolase, which catalyzes the conversion of SAH to homocysteine, a key step in the methionine cycle. Inactivating mutations in this enzyme cause an autosomal recessive disorder that typically presents in infancy with liver disease and developmental delay. A 7-year-old son of the proband, who is healthy and a good student, was found to be homozygous for the same AHCY-R49H mutation. He had mildly elevated aminotransferases and markedly elevated serum levels of methionine, S-adenosylmethionine (SAM), and SAH, confirming that the variant caused a loss of function. The son was placed on a low-methionine diet for 4 weeks, which resulted

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in significant reductions in his aminotransferases, methionine, SAM, and SAH levels. This work reveals that the initial presentation of SAH hydrolase deficiency can be an adult-onset liver disease and that affected women can give birth to healthy children. Serum amino acid measurement should be included in the evaluation and management of patients with liver disease of unknown etiology, especially in those with a family history of consanguinity. Finally, future studies will determine if the strikingly early onset of hepatocellular carcinoma in this patient is due to alterations in DNA or protein methylation secondary to SAH-mediated inhibition of methyltransferases.

HHMI-FFB-14

Reversal of autosomal dominant retinitis pigmentosa using rAAV delivery of CRISPR/Cas9

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Autosomal dominant-inherited diseases pose a unique challenge in gene therapy because they require inactivation of a disruptive allele before a supplemented gene's effects can be propagated. Gene editing using programmable nucleases has become an attractive potential therapeutic because of its ability to permanently knockout gene expression. By subsequently introducing WT cDNA, this "kill and replace" strategy offers a solution to autosomal dominant disease therapy. The eye is a great testbed for optimizing gene therapy because of its relative immunoprivilege and contained environment. Retinitis pigmentosa (RP), the most prevalent hereditary cause of blindness, refers to a diverse collection of inherited retinal degeneration disorders that progressively constrict the visual field, eventually resulting in blindness. Rhodopsin, the primary photon detector protein found in photoreceptor cells, is the most common gene mutated in the autosomal dominant form of RP (ADRP). In this study, we utilize the CRISPR/Cas9 endonuclease system to introduce double strand breaks to investigate rhodopsin-linked ADRP therapy. We package Cas9 and guide RNA's targeting rhodopsin into rAAV8, a serotype of adeno-associated virus with a tropism for photoreceptor cells. Our strategy is to perform subretinal injections of these rAAV8 vectors in heterozygous and homozygous P23H-human rhodopsin-RFP mice along with cotransduction of WT rhodopsin cDNA in the latter mice. Gene editing is confirmed by loss of fluorescence and deep sequencing while morphology is assessed with retinal wholemounts. Ultimately, this study will establish a "kill and replace" strategy that can be applied to a whole range of autosomal dominant-inherited diseases.

HHMI-15

Significance of tubular epithelial density on functional recovery of tubular architecture after acute kidney injury

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After kidney ischemia/reperfusion (I/R) injury, the surviving resident tubular cells proliferate and serve as the principal source of tubule regeneration. While many studies have uncovered the signaling pathways and growth factors critical to this process, little is known about the functional recovery of the tubular architecture and the role that this plays in suppressing or promoting the interstitial scarring that can lead to chronic kidney disease. We hypothesized that there is an optimal cell density required for tubular epithelial cell function, and that this homeostatic set point must be reached again after injury in order for the tubule to re-establish normal architecture and function. To assess these changes, we used two photon microscopy to create and analyze the three dimensional morphology of injured versus non-injured nephron segments in I/R injured C57BL/6 mice. We then used ImageJ to manually reconstruct tubule segments and evaluate their nuclear density. Our preliminary data demonstrates a density of 5.34^{-4} nuclei/μm^3 in the S3 segment of the proximal tubule. To characterize these differences at the transcriptional level, we induced I/R injury in a separate mouse model, *ROSA26-Tomato-mGFP/yGT-Cre* mice in which proximal tubule cells fluoresce green and all other cells fluoresce red. We then utilized Laser Capture Microdissection to specifically isolate proximal tubules for comparison of tubular cell mRNA expression of injured versus non-injured proximal tubules by RNA sequencing. These results will provide new insights into the fundamental drivers of fibrosis and thus identify novel targets for therapies to suppress the transition from acute kidney injury to chronic kidney disease.

HHMI-16

Complete genomic sequences of *Propionibacterium freudenreichii* phages from Swiss cheese reveal more diverse genomes than *Propionibacterium acnes* phages

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Acne is a major dermatological issue, causing not only physical scarring but also significant psychological distress. The pathogenesis of acne has been in part attributed to the role of *Propionibacterium acnes* and the current most effective treatment is oral antibiotics limited by development of resistance and isotretinoin which has many systemic side

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effects. In studying the phage which infect *P. acnes*, as a potential alternative acne treatment, we found these phage genomes to be surprisingly limited in diversity. To determine if this is a feature of the *Propionibacterium* genus, we sequenced the genomes of phages which infect *P. freudenreichii*, present in Swiss cheese. We have isolated and sequenced the full genomes of seven *P. freudenreichii* phages from various cheese samples that fall into two distinct genomic types. Cluster BV includes Anatole, E1, B3 and Cluster BW includes Doucette, B22, E6, G4. Our data demonstrating the genetic diversity in *P. freudenreichii* phages is in striking contrast to high homology of *P. acnes* phages. *P. freudenreichii* phage and host have similar GC content of ~65% and there is presence of prophage sequences in the host, suggesting phage-bacterium interaction at genomic level. These findings suggest substantial differences in the evolution of different *Propionibacterium* spp. The firstly fully sequenced phages also sheds greater understanding in finding ways to control phage contamination in cheese factories. Finally, there is a genetic system for introducing genes into *P. freudenreichii*, unlike *P. acnes*, such that the genome comparisons performed in this study provide insight in creating a shuttle phage vector to introduce the engineered phage to target acne.

HHMI-17

Partner selectivity in Drosophila neural circuits

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As neural circuits develop, selective connections form. The current model states that axons and dendrites are placed using a three-dimensional coordinate system of guidance cues. Once pre- and postsynaptic partners arrive in the appropriate termination domain, connections are made at random; activity determines ultimate connectivity. Local target-derived cues may determine precise connectivity between partners, however the identity and role of these putative cues is unclear. Pre- and postsynaptic partners are not known in most systems; where partners are known, they are difficult to manipulate. The *Drosophila* embryo is an ideal model to search for target-derived cues given the wealth of genetic tools and connectomic reconstructions. The circuitry downstream of somatosensory neurons has recently been characterized in full using serial section electron microscopy (EM) reconstruction. Here we introduce an experimental connectomics approach to test the model of partner selectivity. Through manipulation of receptors for repulsive guidance cues, we selectively shifted pre- or postsynaptic partners to directly test the role of target-derived attractants in the neuropile; the processes of mutant animals with aberrantly placed presynaptic axons and wild type postsynaptic dendrites were then traced with EM reconstruction. We found that wild type postsynaptic interneuron dendrites are able to seek and find their presynaptic sensory axon partners. This suggests that partner-level selectivity cues exist. RNA-seq of pre- and postsynaptic partners revealed over 250 candidate genes; the tetraspanin family makes up over 30 of these candidates and provides the combinatorial logic for a partner-level selectivity code. We are now testing the functional role of the tetraspanins in selective synaptogenesis. Our results suggest that neurons make highly selective connections using a combinatorial molecular code; understanding this code is key to understanding disorders of aberrant wiring.

HHMI-18

Steroid-5α-reductase-1 as a potential target to disrupt progression to castration-resistant prostate cancer

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Androgen deprivation therapy (ADT) by medical or surgical castration is a mainstay of treatment for prostate cancer, the second leading cause of cancer-related death in U.S. men. Although ADT is initially effective in disrupting androgen receptor (AR) signaling necessary for tumor growth, responses to ADT are almost invariably followed by recurrence with lethal castration-resistant prostate cancer (CRPC). We are interested in characterizing non-canonical pathways of androgen synthesis, which can restore AR activity during this pathophysiologic transition. Specifically, our aim is to elucidate the role of steroid-5α-reductase isoenzyme-1 (SRD5A1) in harnessing adrenal-derived steroids to bypass T as an obligate precursor for DHT synthesis. To model potential differences in routes of DHT synthesis in a hormone therapy-naïve versus castrate setting, we first developed a method of ex vivo culture using prostate tissue procured from men undergoing surgery for localized cancer. We then employed stable isotopic tracing with ^{13}C-labeled AD and analysis by liquid chromatography-tandem mass spectrometry to track carbon labeling of downstream metabolites, under varying conditions of T availability. For comparison, metastatic prostate cancer cell lines were similarly studied. Additionally, stable knockdown cell lines with *SRD5A1* silencing were generated for ongoing investigations of SRD5A1-mediated AR signaling and DHT maintenance. Early findings demonstrate that AD contributes to DHT in a non-castrate setting, and AD may even be a preferred substrate in localized, hormone-naïve prostate cancer. Continued work will focus on further elucidating distinct metabolic patterns in localized cancer and potential co-regulatory functions of SRD5A1.

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HHMI-19

Mouse models expressing precursors of broadly neutralizing antibodies against HIV

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Mouse models expressing precursors of anti-HIV-1 broadly neutralizing antibodies (bnAb) could serve as assay systems for vaccine candidates. The conventional approach is to integrate assembled human immunoglobulin (Ig) V(D)J exons into mouse Ig loci. However, this approach generates a limited primary antibody repertoire. Also, owing to poly-reactivity of certain bnAbs, immature B cells expressing them may be selected against. We devised strategies to address such impediments via a system that involves introducing Ig locus modifications into ES cells and then introducing them directly into B cells via Rag^2 deficient blastocyst complementation. To address diversity, we separate bnAb Ig genes into component gene segments, incorporate the segments into normal locations in mouse Ig loci, and let them reassemble via V(D)J recombination in developing B cells. In this setting, the mice produce a diverse range of primary bnAbs variants, some of which also may not impede B cell maturation. To optimize this approach, we deleted the IGCR-1 *IgH* regulatory element to promote the high level usage of the human V_H segment which is inserted in place of the proximal mouse V_H segment. By this approach we have generated a mouse model for VRC01 bnAb. A unique aspect of our model is that, analogous to physiological situations, a specific V segment for this bnAb family (IGHV1-2*02) is expressed in association with diverse CDR3's. In the second approach, we drive B cell development with Ig V(D)J exon that is not self-reactive and use the cre-loxP recombination system to swap the "driver exon" with the bnAb V(D)J exon in mature B cells. By this method, we expressed the VRC^{26} bnAb that features an unusual antigen-binding site and which is selected against during B cell development.

HHMI-20

Testing inhibitors of NRAS-associated pathways as potential therapeutic strategies for preclinical models of congenital giant nevi

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Giant congenital melanocytic nevi are oncogene-driven proliferations of melanocytes present since birth that are greater than 20 cm in projected adult size, and have a melanoma conversion frequency of approximately 10 to 15%. Melanoma in these patients typically develops during childhood, is extremely aggressive, is refractory to therapy, and is almost universally fatal. Therefore, targeted therapies would be enormously beneficial. Neuroblastoma RAS Viral Oncogene Homolog (NRAS) activating mutations are postulated to be the driver mutation of giant congenital nevi. I wish to test the hypothesis that constitutive NRAS activation may be important for maintenance of congenital melanocytic nevi, and that inactivation of pathways constitutively stimulated by this mutation may provide a therapeutic opportunity for children with these lesions. For *in vivo* studies, we established two giant congenital nevi murine models: one with an inducible NRAS activating mutation *($Tyr::CreER^{T2};NRAS^{Q61R}$)*, and one with a constitutive mutation *($Dct::Cre;NRAS^{Q61R}$)*. For *in vitro* studies, we established corresponding primary cell lines from our two murine models. We will also establish melanoma cell lines from melanomas arising from our mouse models. These models serve as a useful toolkit for screening drug therapies that may aid in congenital giant nevus regression. Current therapies being screened include targeted small molecule inhibitors, epigenetic modifiers, melanocytotoxic agents, immunotherapy drugs, and combination therapies. Efficacies of molecules in the *in vitro* drug screen will be evaluated by growth curve assays, and effective therapies will then be tested in the *in vivo* model. To test drug efficacy in the *in vivo* model, we will take punch biopsies of the nevus lesion to visualize skin architecture and evaluate nevus regression. Hopefully, utilizing these several novel preclinical models will lead to potential clinical treatment alternatives to the current limited options.

HHMI-21

Osteogenic gene expression and cell proliferation in heterotopic ossification derived cells

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Heterotopic ossification (HO) is the ectopic formation of bone in soft tissue, often caused by trauma or due to rare genetic mutations in BMP-SMAD pathways. HO can cause pain, nerve entrapment, and limit mobility. Treatments include pharmacologic and surgical modalities but are limited by residual deficit or recurrence. Modulation of osteogenic signaling is a proposed therapeutic avenue, however, it remains unclear if HO associated cells demonstrate differences in osteogenic signaling compared to cells in uninjured native tissues, which could inform and substantiate novel treatments. Here, we evaluate the growth and osteogenic capacity of both murine HO, via a trauma-induced model and a genetic model (*$Nfatc1\text{-}cre/caACVR1^{fl/wt}$*), as well as human HO. We then evaluate the response of these cells to the small molecule BMP receptor inhibitor LDN-212854. We hypothesize that cells derived from HO will demonstrate increased proliferation and up-regulation of osteogenic mRNAs and BMP-SMAD proteins that will be mitigated by LDN-212854.

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Cells were isolated from HO and from corresponding native tissues in each model. Cell proliferation was assessed by BrdU ELISA. Osteogenic differentiation was assessed by alkaline phosphatase (ALP) and alizarin red (AR) stain, and mRNA and protein expression. In all three models HO-derived cells showed greater proliferation ($p<0.05$), ALP, and AR staining, and pSmad 1/5 expression versus control cells. Both murine models showed increased mRNA expression for *alp, ocn, osx,* and *runx2*. Inhibition of osteogenic signaling via LDN-212854 decreased osteogenic capacity by ALP and AR quantification ($p<0.05$), but interestingly not to native levels. These data demonstrate that cells derived from HO exhibit significant differences in cellular behavior and osteogenic capacity compared to native cells and contribute to rationale for the use of therapeutics targeting osteogenic pathways.

HHMI-22

A programmed death pathway in the axon

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Axon degeneration is a form of programmed subcellular death resulting in the destruction of axons in injury and disease states. Injury-induced axon degeneration is thought to be independent of classic apoptotic regulators and necroptotic pathways as genetic and pharmacologic inhibition of these pathways do not significantly delay or prevent degeneration. Sterile alpha and TIR motif- containing-1 (SARM1) was recently reported to be an essential mediator of an injury-induced axon death pathway, though the mechanism behind its action remains to be solved. Here, we report that SARM1 mediates axon degeneration locally, by a catastrophic depletion of nicotinamide adenine dinucleotide (NAD^+). First, we demonstrate that dimerization of the TIR domain of SARM1 using a pharmacologically controlled dimerization system, is sufficient to induce axon degeneration. Moreover, catastrophic loss of NAD^+ within 15 minutes after TIR dimerization underlies this degeneration process. Finally, supplementation with the NAD precursor, nicotinamide riboside (NR), and overexpression of the NAD^+ biosynthetic enzyme, nicotinamide mononucleotide adenylyltransferase (NMNAT) significantly delay this active degenerative process. Ongoing studies include using CRISPR/Cas9 genome editing of known NAD-consuming enzymes like poly (ADP) ribose (PARP), sirtuin, and CD38, to identify the enzyme responsible for NAD depletion upon TIR dimerization. These studies will help elucidate the molecular underpinnings of axon degeneration, and provide new targets for neurodegenerative diseases.

HHMI-AGA-23

High serum soluble intercellular adhesion molecule-1 concentration (sICAM-1) is associated with hepatocellular carcinoma development in patients with chronic hepatitis B, chronic hepatitis C, or non-viral liver disease: multiplex analysis of 51 cytokines and other serum markers

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Hepatocellular carcinoma (HCC) is a serious complication of chronic liver disease and a leading cause of cancer mortality worldwide. Conventional risk factors such as cirrhosis, age, and ethnicity have limited ability to predict HCC risk. Our goal was to identify serum markers that can predict HCC development in patients with underlying liver disease. We performed a prospective cohort study of 284 patients with chronic liver disease (106 hepatitis B virus [HBV], 114 hepatitis C virus [HCV], and 64 non-viral) without HCC at baseline. Patients were enrolled from 2000 to 2007 and followed until HCC development (median follow-up=155.3 months, IQR=45.8-210.8 months). Serum samples were obtained at baseline, and microplex analysis (Luminex 200 IS) was used to measure serum levels of 51 common cytokines and other biomarkers. Lasso regression showed soluble intercellular adhesion molecule 1 (sICAM-1) to be the most informative serum marker for HCC development. Patients with baseline sICAM-1 level above the median (>11,000 pg/mL) had significantly higher HCC incidence than those with below - median levels ($p=0.001$). At a cutoff of 13,000 pg/mL, the association between sICAM-1 and HCC risk was strongest and most significant (hazard ratio [HR]=4.38; $p<0.0001$). For each individual etiology subgroup, there was at least a trend toward an association between high serum sICAM-1 concentration and increased HCC incidence, though these were not always statistically significant ($p = 0.027$, 0.133, and 0.033 for HBV, HCV, and non-viral, respectively). On multivariate analysis including age, sex, and cirrhosis status, sICAM-1 remained associated with HCC risk (HR=2.50, 95% CI=1.09-5.74, $p=0.031$). Higher serum sICAM-1 concentration is associated with significantly higher risk for HCC development, independent of conventional clinical risk factors and across diverse etiologies of liver disease.

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HHMI-24

Stromal interaction molecule 1 (STIM1) is required for a normal contractile phenotype in the murine myometrium

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Appropriate regulation of uterine contractility is necessary for successful parturition. Store-operated calcium entry (SOCE) is the pathway by which cytosolic calcium $[Ca^{2+}]i$ is replenished in response to depleted calcium stores in the sarcoplasmic reticulum (SR). Stromal interaction molecule 1 (STIM1) is a single-pass transmembrane protein that acts as a $[Ca^{2+}]$ sensor by activating SOCE channels on the plasma membrane in response to depleted SR $[Ca^{2+}]$. The objective of this study was to determine the extent of STIM1 expression and function in the myometrium. C57BL/6J (B6) mice underwent timed matings and myometrium was harvested at various time points during the pregnancy and processed for histology, western blot, and electron microscopy. Next, myometrial strips from non-pregnant and term pregnant B6 mice were suspended in a tissue organ bath and stimulated with increasing doses of oxytocin in the presence of a STIM1 inhibitor or vehicle control and the contraction responses measured. Lastly, myometrial strips from a STIM1-LacZ haploinsufficiency model were harvested for staining as well as contraction assays in a tissue organ bath. Contraction responses were compared using two-way repeated measures ANOVA with corrections for multiple comparisons. STIM1 is expressed in the murine myometrium and localizes to the SR and plasma membrane. STIM1 is upregulated during early pregnancy and remains elevated throughout gestation and immediately postpartum. Treatment of uterine muscle strips with a SOCE inhibitor results in decreased contraction force and frequency in response to oxytocin in both pregnant and non-pregnant mice. Similarly, myometrial strips isolated from a genetic model of STIM1 haploinsufficiency also demonstrated decreased uterine contraction force and frequency in response to oxytocin compared to controls. Finally, both STIM1 pharmacologic inhibition and STIM1 haploinsufficiency result in an inability to produce tetanic contractions in response to oxytocin. STIM1 is critical in tissues that undergo repetitive contraction. STIM1 is necessary for a normal contractile phenotype in the myometrium of both pregnant and non-pregnant mice. Variations in STIM1 genotype or expression within the myometrium of women may result in dysfunctional labor phenotypes.

HHMI-25

Characterizing the innate-like B-1 cell in a murine model of ischemia reperfusion injury

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We propose a reexamination of the underappreciated role of B-1 cells, an innate-like subset of B lymphocytes, in ischemia reperfusion injury (IRI). IRI is a two phase insult composed of an acute, ischemic injury leading to energy depletion and oxidative species which, alongside cellular effectors, cause further damage following reperfusion, resumption of blood flow. IRI takes place in a variety of clinical settings but is particularly relevant to transplant recipients since every allograft will experience some degree of IRI. B-1 cells are a subset of B lymphocytes different from conventional, B-2 cells, in that they derive from a self-renewing progenitor early in development. They do not undergo N region addition during V region rearrangement; nor do they undergo somatic hypermutation and thus exhibit a limited repertoire of lower affinity natural antibody (NAb). A common target of NAb are self-antigens associated with apoptotic debris. CD5 expression distinguishes B-1a(+) and B-1b(-) cells. We established a model of IRI through unilateral renal pedicle clamping for 60 min. In B6 mice, following IRI, there is an increase in B-1a cells by FACS as early as 16 hours compared to shams (300%, $p<0.05$), and is maintained in the inflamed tissues at least through 72 hours. *In vitro*, these cells possess many regulatory functions including the potential to produce IL-10 in response to BCR signaling. At 72 hours, there is an increase in T lymphocytes in the inflamed kidney (250%, $p<0.05$). Preliminary experiments indicate ~15% of the T cell infiltrate is CD4+CD25+, possibly Treg. We propose to examine whether B-1a cells have a regulatory role *in vivo* and if so do they interact with Treg. We are also performing heterotopic cardiac transplants to evaluate the impact of these cells on peripheral tolerance. Understanding how inflammation is regulated following ischemia and the role of B-1a cells in clinically relevant transplant models will provide new therapeutic approaches for transplant recipients.

HHMI-26

Intravascular magnetic enrichment of circulating tumor cells

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Metastatic cancer is likely mediated by circulating tumor cells (CTCs). Enumeration and analysis of CTCs allows insight into both a tumor's presence and its molecular profile, allowing earlier detection and more effective guidance of therapy.

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However, CTCs are extremely rare, often found at levels of only 1-10 cells per ml of blood. Current detection methods usually capture CTCs from a standard blood draw of 7.5 ml, and the low CTC yield is often inadequate for molecular characterization of disease. Here we describe a minimally-invasive device to enrich and retrieve CTCs from the entire subject blood volume (5 L in an adult), thereby greatly increasing the number of CTCs that can be detected and characterized. A flexible wire composed of neodymium magnets < 1 mm in diameter would be inserted intravascularly into a patient's arm. The patient would be injected with 1 µm magnetic iron oxide nanoparticles that have been functionalized with antibodies against EpCAM, a common surface marker on CTCs, in order to bind CTCs in the bloodstream and allow them to be captured on the magnetic wire. After the CTC-coated wire is retrieved, the captured cells can be characterized by PCR or cultured for drug-resistance testing. The device captures > 50% of circulating cells in spiking experiments within a closed-loop circulation system, and > 15% of cells after only a single pass. When sampling the entire blood volume over 1 hour, this would translate into a roughly 100-fold increase in yield compared to traditional techniques. We aim to eventually capture CTCs shed from endogenous tumors in rabbits. This work is a first proof-of-principle of intravascular CTC detection, and also has applications to other circulating biomarkers in the blood.

HHMI-27

In vitro and *in vivo* characterization of a novel human SIRP-α agonist antibody

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Signal regulatory protein alpha (SIRP-α) is a membrane receptor whose expression is mostly restricted to phagocytic hematopoietic cells whereas its ligand, CD47, is virtually expressed on every mammalian cell. When bound to CD47, this interaction paralyzes phagocytic cells from ingesting their targets, and thus this binding has been described as a "don't eat me" signal. Since many cancers have been shown to up-regulate CD47, blocking this signaling axis has significant therapeutic potential by promoting phagocytosis of tumor cells. However, enhancing this signal to mitigate phagocytosis for the treatment of autoimmune disease has been understudied due to a lack of agonistic agents. Here we describe a novel agonist antibody, P362, that binds human SIRP-α. P362 is a fully humanized monoclonal antibody with an IgG4 isotype. We show that P362 can outcompete soluble human CD47 binding to human SIRP-α in a dose dependent manner. We also tested the antibody's ability to reduce *in vitro* phagocytosis using human macrophages derived from donor peripheral blood mononuclear cells. P362 significantly decreased cetuximab-induced phagocytosis of an EGFR expressing colon cancer cell line, DLD-1. Similarly, P362 significantly inhibited rituximab-induced phagocytosis of a CD20 expressing lymphoma cell line, Raji, by primary human macrophages. We also tested the therapeutic potential of P362 for treating autoimmune hemolytic anemia, where macrophages pathologically phagocytose erythrocytes coated by autoantibodies. *In vitro*, P362 was able to reduce donor macrophage phagocytosis of opsonized human erythrocytes by 40-50%. Additionally, in transgenic mice bearing human SIRP-α, P362 was able to extend the half-life of injected human erythrocytes by 3.3-fold. We believe P362 is a new tool that will enable us to further study the CD47- SIRP-α axis and represents a potential therapeutic agent.

HHMI-28

A convection-enhanced macroencapsulation device for the treatment of type I diabetes

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Subcutaneously-implantable, immunoisolated macroencapsulation devices (MEDs) restore glucose homeostasis in rodent models of type 1 diabetes (T1D). These pouch-like devices protect transplanted β-cells from the host's immune system, thereby obviating insulin injections and immunosuppression. Despite their promise, current MEDs have yet to reach the clinic because they cannot sustain enough cells. The problem is dependence on passive diffusion to harvest nutrients from the surrounding host tissue, which is only sufficient to feed a monolayer of islets housed in a wafer-thin capsule (a 0.4 cm^2 device supports ~1,000 islets). Because >500,000 islets are required to maintain euglycemia in a human adult, a T1D patient would require >500 wafer devices, making diffusion-limited MEDs impractical therapies. Many biotech companies have attempted to improve diffusion-limited MEDs, but no 2D MED geometry has overcome the cell-number limitations preventing clinical efficacy. A MED capable of sustaining physiologic numbers of islets could change the standard of care for type I diabetics. We hypothesize that improving nutrient transport to encapsulated cells will generate such a clinically relevant device. We propose a 3D, convection-enhanced MED (ceMED) capable of perfusing cells beyond the 200 µm diffusion limit, thereby enabling sufficient numbers of islets to be housed in devices practically sized for humans. To test this hypothesis, we created in silico and *in vitro* ceMED models, which demonstrate increased cell survival and glucose-stimulated insulin secretion as perfusion rate increases. We determined the necessary dimensions and perfusion rates of effective ceMEDs

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for T1D therapy and fabricated prototypes for *in vivo* study. We are currently characterizing survival and function of these prototypes in rat models of T1D. As the culmination of this proof-of-concept, we aim to validate a human-sized ceMED that may be accelerated to clinical trials in the next couple of years.

HHMI-29

A new reporter tool: illuminating activation of toxin-antitoxin systems in bacteria

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The emergence of bacterial resistance has increased the number of infections that are difficult to treat. Further complicating matters is bacterial persistence, a noninherited, reversible state where bacteria can transiently survive antibiotic treatment. Recently, toxin-antitoxin (TA) systems, which are co-operonic gene modules widely present on bacterial chromosomes, have been implicated in persister cell formation. TA systems typically encode a toxin that targets essential cellular functions and an antitoxin that binds and neutralizes its cognate toxin. Further, the TA complex inhibits its own transcription. For a toxin to become activated, the intrinsically more labile antitoxin typically must be degraded even faster, resulting in increased transcription and allowing the unbound toxin to inhibit growth. The current model for TA system activation suggests a linear pathway in which an increase in the small-molecule (p)ppGpp results in build up of inorganic polyphosphate, which then activates the Lon protease that degrades antitoxins and activates TA systems. However, this model remains largely untested. We developed a new method that monitors TA system activation *in vivo* through a transcriptional reporter. We found that overproducing (p)ppGpp elevates signal from a reporter for the *relBE* system, both in the presence and absence of RelBE. We also observed reduced *relBE* reporter activity in cells lacking Lon, again, in the presence or absence of RelBE. The RelBE-independent effects of (p)ppGpp and Lon on the *relBE* promoter challenge the current model in which (p)ppGpp activates TA systems by promoting Lon degradation of antitoxins. Lastly, we showed elevated reporter signal of the *mazEF* TA system in cells treated with sublethal amounts of antibiotic, a condition thought to generate persister cells. Insights gained from our reporters will provide a better understanding of TA systems and their potential contribution to persistent bacterial infections.

HHMI-30

A novel reporter of B cell auto-reactivity and senescence

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Although humoral immunity is impaired with advanced age, autoantibody production paradoxically increases with age. The mechanisms linking these phenomena with the senescence of the B cell compartment, however, remain poorly understood. While bone marrow B cell production declines markedly in aged mice, the total number of mature naive B cells surprisingly remains stable. Interestingly, *in vivo* labeling studies revealed that mature B cells in aged mice have a several-fold increase in life span to account for their persistence in the face of declining bone marrow output. Neither the cause nor the consequences of this increase in B cell lifespan have been elucidated. A means of marking the relative age of individual mature naïve B cells could serve as a powerful tool to explore the impact of cellular age on gene expression, antigen receptor repertoire, and functional capacity to participate in protective and pathologic immune responses. Microarray studies by Goodnow and colleagues probing the genetic basis of B cell anergy identified *Crisp-3*, a gene of elusive function, to be highly upregulated in MD4xML5 anergic B cells compared to MD4 "naïve" B cells. Here we report the characterization of a novel reporter mouse line in which GFP is knocked-in to the *Crisp-3* genomic locus. GFP expression in this system is restricted to late transitional (T3) and mature follicular B cells. Reporter expression is not directly induced by BCR signaling, but requires endogenous antigen encounter, and marks auto-reactive B cells with low surface IgM BCR expression *in vivo*. Unexpectedly, the proportion of GFP-positive naïve B cells increases from 20% at age 7 weeks to 80% at age 9 months. The Crisp3 reporter thus marks an aging-associated feature of the B cell repertoire that may reflect heterogeneity at the level of B cell half-life, auto-reactivity, and/or gene expression. Ongoing efforts are aimed at characterizing phenotypic and functional differences between GFP-positive and GFP-negative reporter B cells.

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HHMI-OREF-31

Phlpp inhibitors may reduce pain and be potential candidates for osteoarthritis treatment

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Osteoarthritis (OA) is the most common form of degenerative joint disease in the United States. OA poses an immense social and financial burden as it affects more than 27 million people and costs over $43 billion on healthcare annually. Current treatment regimens lack an FDA approved disease modifying drug and consist only of palliative measures, which do not change the disease progression, or radical joint replacement surgeries. It is imperative that we find therapeutic targets that can directly affect OA development. We previously identified pleckstrin homology domain leucine-rich repeat protein phosphatase (PHLPP) as a potential treatment target. PHLPP is a protein phosphatase that suppresses chondrocyte proliferation and cartilage matrix production. PHLPP expression was found to be increased in human OA tissue and Phlpp1 knockout mice were more resistant to surgically induced OA changes. PHLPP inhibitor NC117079 was tested on cell and animal models. In ATDC5 chondrocytes, 5 μM NC117079 significantly increased Akt and PKC phosphorylation and cartilage matrix production. 12-week-old male C57 mice underwent sham surgery (control) or surgery to destabilize the medial meniscotibial ligament (DMM), which induces OA changes by week 6. At 7 weeks following sham or DMM surgery, mice received an intra-articular injection of PBS or 8μM NC117079. The Phlpp inhibitor did not shown adverse reaction or changes in activity and pain in a surgical sham group, establishing the safety of NC117079. The DMM group showed increased pain by 6 weeks as measured by von Frey assays using 0.16g, 0.4g, and 0.6g filaments, indicative of OA development. When the von Frey measurements were taken again at 1-week post-injection, the Phlpp inhibitor group showed decreasing trend in response to pain. Activity remained unchanged between PBS and inhibitor injected groups. Results of ongoing pain, histology, and imaging studies that further evaluate the effects of Phlpp inhibitor on OA progression will be presented.

HHMI-32

The role of red blood cells in reverse cholesterol transport

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Cholesterol is transported from peripheral tissues to the liver through the blood, where it is secreted into bile. Plasma high density lipoproteins (HDL) have been proposed as a major vehicle for this process; however, mice lacking HDL do not accumulate cholesterol in peripheral tissues. This finding indicates that another blood component can transport cholesterol to the liver. We propose that red blood cells (RBCs), which contain 50% of the cholesterol in blood, transport cholesterol to the liver and that hepatocytes take up cholesterol from RBCs via scavenger receptor class B type 1 (SCARB1). To test this hypothesis, we established an assay to measure the amount of cholesterol in RBC membranes that is accessible for uptake by SCARB1. Accessible RBC cholesterol is quantitated using a fluorescently labeled protein (fALOD4) that specifically binds cholesterol in intact cell membranes. We have found that RBC-fALOD4 binding varies 10-fold among individuals and is not related to the total cholesterol content of the RBC membrane. As we further explore the etiology and clinical significance of differences in RBC-fALOD4 binding, we are using genetically-modified mice to test the hypothesis that RBCs play a role in cholesterol transport. We have found that fALOD4 binding is very low in mice expressing no ApoA1 and thus lacking HDL, and is much higher in mice lacking hepatic SCARB1, which have very high HDL levels. To exclude the possibility that RBC-fALOD4 binding simply reflects plasma HDL levels, we are measuring fALOD4 binding in mice lacking both hepatic SCARB1 and ApoA1. We will also use stable isotope labeling to determine the relationship between RBC cholesterol availability and RBC cholesterol flux in these mouse models. Taken together, these studies will further define the role of RBCs in cholesterol transport and may identify another risk factor for atherosclerotic disease.

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HHMI-33

Evaluation of a novel bioengineered hydrogel for intramyocardial delivery of neuregulin-1β to reverse post-infarction cardiac remodeling

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Neuregulin-1β (NRG) is a growth factor and ligand of the ErbB2/ErbB4 receptor that is a key inducer of cardiomyocyte proliferation. Our laboratory has developed a hydrogel platform that enables sustained intramyocardial delivery of NRG. We are now evaluating its therapeutic potential in a rodent model of chronic ischemic cardiomyopathy. Adult male Wistar rats (n=29) underwent permanent ligation of the left anterior descending artery, resulting in a large anterolateral myocardial infarction. Four weeks after infarction, the left ventricle dilated and left ventricular ejection fraction was reduced to 46.2±11.8%, confirming the development of heart failure. Rats subsequently underwent redo thoracotomy, and were randomized to 3 groups: a 100 µL borderzone injection of PBS (n=9), hydrogel alone (n=9), or NRG encapsulated in hydrogel (n=11). Four weeks after treatment, no significant difference in left ventricular dimensions or function was noted between groups. We plan to complete our functional assessment of NRG by increasing sample size. We will also investigate whether NRG is capable of inducing cardiomyocyte proliferation in the setting of chronic heart failure by examining hearts histologically 6 days after treatment, as well as analyzing cardiomyocytes for downstream activation of the ErbB2/ErbB4 signaling pathway. Furthermore, an *in vitro* assessment of NRG's ability to stimulate cardiomyocyte division in human IPS-derived cardiomyocytes is underway.

HHMI-K-RITH-34

Targeting energy metabolic pathways simultaneously to cause synergistically bactericidal effects in *Mycobacterium tuberculosis*

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Mycobacterium tuberculosis (Mtb) is now ranked alongside HIV as the leading cause of death among infectious diseases. *Mtb* is an obligate aerobe that requires the use of flexible energy metabolic pathways to grow and survive. It was shown that when bedaquiline (BDQ), the first new anti-TB drug in 40 years, is used with other energy targeting drugs, clofazimine (CFZ) and Q203, one could achieve synergistic killing both *in vitro* and *in vivo* (infected macrophages) without affecting host cells. Therefore, these data suggest that targeting several energy metabolic pathways simultaneously (i.e. glycolysis, tricarboxylic acid cycle, etc.) that feed into the electron transport chain will cause a greater bactericidal effect. In this study, we optimized the Extracellular Flux Analyzer XF96 to measure the bioenergetics of *in vitro* and *ex vivo Mtb* bacilli. We characterized several new energy pathway-targeting compounds for their efficacy against wildtype *Mtb* grown *in vitro* or isolated *ex-vivo*, and selected resistant mutants thereof, both in single or multi-drug combinations. We further investigated the mechanism of action of the most effective combinations using various techniques including bioenergetic profiling, metabolite analysis, NAD/NADH ratios and ATP/ADP ratios. We have shown that targeting *Mtb* energy pathways hold great promise as an effective drug strategy. Importantly, we propose that *Mtb's* flexible energy metabolism can be turned against itself. Although there are limitations of targeting single components of *Mtb's* energy pathways, we believe they can be overcome by targeting several energy metabolic pathways simultaneously in a combination therapeutic approach.

HHMI-35

Exploring axonal dynamics during neocortical laminar innervation

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The cerebral cortex is a highly interconnected neural network of highly specialized cells within the mammalian brain that facilitates higher cognitive functions. Because discrete processing centers within the cerebral cortex perform complex computations and drive our unconscious and conscious experience of the world, the study of the molecular framework underlying these interconnected neural networks is essential to understand how the brain functions and the molecular basis of cognitive disorders. Therefore, our goal is to study how extracellular and intercellular molecular signaling cascades drive select innervation events during brain development. To approach this task, we focused on a group of neurons involved in connecting and integrating information from different functional areas of the cerebral cortex: layer II/III pyramidal neurons. Many of these neurons form cortico-cortico neural circuits that exhibit a highly stereotyped innervation pattern. To better understand how these cells ultimately form these cortico-cortico networks, we visualized the developing innervation patterns of layer II/III pyramidal neurons located in the primary somatosensory cortex. Once we established the precise timing of innervation by layer II/III pyramidal neurons, we explored developmental mechanisms underlying this stereotypic innervation pattern and found that

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layer II/III cortical layer-specific innervation is independent of intrinsic cellular activity, thalamocortical input, and central nervous system midline crossing. Furthermore, we performed time-lapse microscopy on developing layer II/III pyramidal neurons and analyzed the axon branching and cytoskeletal dynamics responsible for laminar-specific innervation. To better visualize the developing mammalian cortex and to enable the study of complex cerebral cortex connectivity in the context of a whole brain, we developed a rapid brain clearing technique that facilitates our studies, and that should be of use to the neuroscience community at large. To identify the genetic and molecular basis of layer II/III pyramidal neuron connectivity, we are currently performing a targeted screen to elucidate the underlying molecular cues that direct layer II/III pyramidal neuron axon branching patterns during development.

HHMI-36

Whole exome sequencing reveals patterns of recurrent mutations in atypical chronic myeloid leukemia and chronic neutrophilic leukemia

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Atypical chronic myeloid leukemia (aCML) and chronic neutrophilic leukemia (CNL) are rare hematologic malignancies characterized by overlapping clinicopathologic features, including leukocytosis and a hypercellular bone marrow. While oncogenic mutations in colony stimulating factor 3 receptor *(CSF3R)*, a prominent receptor involved in neutrophil function and differentiation, are commonly reported in aCML/CNL, there remain a significant percentage of cases in which the molecular pathogenesis remains poorly defined. We performed whole exome sequencing of leukemia samples from 86 patients with suspected aCML/CNL and identified at least one somatic mutation associated with myelodysplasia or myeloid malignancies in 74 (86%) samples, including 20 samples with *CSF3R* mutations. Segregation of these mutations according to *CSF3R* mutation status revealed that patients with *CSF3R* mutations had a higher frequency of gain-of-function mutations in *SETBP1*, which normally inhibits the tumor suppressor protein phosphatase 2A, compared to patients without *CSF3R* mutations (55% in *CSF3R* mutant samples vs. 24% in *CSF3R* wild-type samples; p=0.02). Mutations in the tumor suppressor *TP53* occurred exclusively in *CSF3R* mutant patient samples, while variants in JAK2, a direct downstream target of *CSF3R*, occurred exclusively in *CSF3R* wild-type samples. Mutations in *ASXL1*, a chromatin-binding protein, occurred more frequently in *CSF3R*-mutant samples (30% in *CSF3R* mutant samples vs. 15% in *CSF3R* wild-type samples; p=0.20). Collectively, these results suggest that aCML/CNL patients with *CSF3R* mutations are also likely to harbor mutations in *SETBP1* and *TP53*. Additionally, because JAK2 is a direct downstream target of *CSF3R*, our results suggest that only one mutation in this signaling pathway is necessary for leukemogenesis. To our knowledge, this is the first comprehensive whole exome sequencing-based study of aCML/CNL and aids in defining the genetic landscape of these leukemias.

HHMI-37

Single-cell RNA-seq analysis of bicuspid aortic valve development in Nos3$^{-/-}$ mice

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Bicuspid aortic valve (BAV) is the most common congenital heart defect, but its developmental mechanism is unknown partly due to the complex interplay of multiple cell lineages in valvulogenesis. The aortic valve arises when the outflow tract (OFT) myocardial cells induce the adjacent endocardium to undergo an epithelial-to-mesenchymal transition (EMT) at E9.5. We used single cell RNA-sequencing to study the cellular heterogeneity of normal and BAV valvulogenesis using Nos3$^{-/-}$ mice, which develop BAV by E11.5. We isolated single OFT cells at E9.5, E10.5, and E11.5 in wild type (WT) and Nos3$^{-/-}$ mice. Cell identity was determined using principal component analysis (PCA), concordant with known cell markers. In WT cells, we found myocytes exhibit a dynamic temporal regulation of cushion formation (*Bmp4, Tgfb2, Vcan, Postn*), and identified endothelial and fibroblast cells undergoing EMT based on expression of *Snai1, Slug,* and *Twist1*. Comparison of WT and Nos3$^{-/-}$ endothelial and fibroblast cells showed differential expression in genes known to regulate EMT (*Id2, Sox9*) and cardiac neural crest cell migration (*Ets1,Sox11*), suggesting dysregulation of these processes in BAV. However, myocardial WT and Nos3$^{-/-}$ cells were indistinct, indicating the Nos3$^{-/-}$ signaling defect is cell lineage-specific. Thus, we showed the temporal regulation of cushion formation by myocytes, delineated transcriptional profiles of early valve interstitial cells undergoing EMT, and proposed specific processes leading to BAV formation. Further dissection of the dynamic intercellular dialogue underlying these disrupted pathways will aid our understanding of BAV.

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HHMI-BWF-38

The architecture of the Flavivirus 3 prime untranslated region, pathogenic RNA production, and viral cytopathicity

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Flaviviruses (FVs), which include Dengue, West Nile, and Yellow Fever, are growing global threats with no available anti-viral therapy. In an FV infection, a viral non-coding RNA is produced to high levels. This subgenomic Flaviviral RNA (sfRNA) has been directly linked to viral cytopathicity. sfRNAs are produced when a host cell's exoribonuclease, Xrn1, degrades the viral RNA in the 5 prime to 3 prime direction, but stalls at specific sites in the viral RNA's 3 prime untranslated region (UTR) and cannot complete degradation. The structures that halt Xrn1 are Xrn1 resistant RNAs (xrRNAs) and the multiple xrRNAs in each FV result in production of sfRNAs of specific lengths and quantities. Biochemical techniques can be used to investigate the kinetics of Xrn1-mediated RNA degradation in the context of different FV RNA constructs, including West Nile and Dengue viruses. To examine this, we developed an *in vitro* assay using radiolabeled RNAs to quantitate resistance to Xrn1 over time, analyzed using denaturing polyacrylamide gel electrophoresis to identify degradation products based on relative size. We are using virological and cell culture techniques to examine the cytopathic effects of the FVs in both human and mosquito cell lines. Viral effects differ in human versus vector cells, as vectors remain unaffected by overt clinical signs. The goal is to understand how the identity, pattern, and location of xrRNAs produce specific sfRNAs, and how the pattern of sfRNAs affects the cytopathicity of the virus to identify targets for therapeutic intervention.

HHMI-39

Characterizing the role of PP2A A-alpha mutations in cancer progression

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Advances in the development of targeted therapies has shifted our clinical approach to cancer; where more traditional histological based classification has been replaced with a molecular annotation of the disease. This allows for an integrated treatment approach where tumor genotype is paired in a clinically actionable manner with specific targeted therapies to maximize efficacy and minimize toxicity for each patient. Our laboratory has been involved in the development of a novel series of small molecule activators of PP2A (SMAPs). Protein phosphatase 2A (PP2A) is a tumor suppressor which negatively regulates multiple molecular oncogenic drivers common to a broad range of human malignancies including MYC, AKT, and ERK. In cancer, PP2A is inactivated through several mechanisms including increased expression of endogenous inhibitors, post-translational modifications of the catalytic subunit, and somatic mutation in its catalytic, regulatory or scaffold subunits. The most frequent somatic PP2A mutations occur in the Aα scaffolding subunit of the protein. The structural and biological basis for PP2A inactivation by these Aα mutations has yet to be fully elucidated. The structural Aα subunit of PP2A is mutated in 2.2% of lung adenocarcinoma patients, with mutations occurring within crucial interaction regions responsible for regulatory subunit binding. We hypothesize that these mutations contribute to oncogenic progression in lung adenocarcinoma and may affect the therapeutic potential for pharmaceutical PP2A reactivation by SMAPs. To test these hypotheses, we generated H358 and H441 KRAS driven lung adenocarcinoma cell lines harboring single site mutant PP2A, including *W140C, R183W,R258H,* or *A541G*. Preliminary data demonstrates alterations of *in vivo* growth parameters and unique aberrations in regulatory subunit binding in each mutant cell line. This data will help characterize the biologic role of PP2A mutations in lung adenocarcinoma tumorigenesis and identify biomarkers for patient response to our novel SMAP therapies.

HHMI-40

Deconstructing interactions between macrophages and hematopoietic stem cells in the embryonic niche

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The hematopoietic stem cell (HSC) niche is a specialized microenvironment that supports the maintenance and regulation of HSCs. Studies of mammalian bone marrow have suggested a retention function for macrophages in the HSC niche, but the underlying molecular mechanisms remain poorly understood. The zebrafish embryo has emerged as an excellent model to study the HSC niche where cells can be directly visualized *in vivo*. Using transgene reporters labeling HSCs (CD41:GFP) and macrophages (mpeg1:mCherry), we have observed intimate cell-cell interactions between macrophages and newly arriving HSCs in the caudal hematopoietic tissue (CHT) niche. As nascent HSCs enter the CHT, macrophages interrogate and groom the HSC surface. From 36-54 hours post fertilization (hpf), 20% of CD41 cells in the CHT interact with macrophages at steady state. We hypothesize that these interactions may induce changes to the

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HSC that promote engraftment. To identify surface molecules mediating these interactions, we performed RNA-seq on isolated macrophages and generated a ranked list based on expression levels of genes (FPKM values). We also included genes predicted to have a transmembrane domain. In our current studies, we are using a macrophage-specific CRISPR/Cas9 system to disrupt candidate genes, including beta-integrins such as itgb2, itgb7, and itgb1b. To assay engraftment, we are then using live cell imaging to quantify the frequency of macrophage-HSC interactions and enumerate HSCs in the CHT at 72 hpf. We have also established *in vitro* co-cultures with sorted HSCs and macrophages to study these interactions in a more controlled environment. In preliminary experiments, 25% of HSCs are engaged by a macrophage at steady state, paralleling *in vivo* data. In contrast, only 5% of erythrocytes (LCR:GFP) are engaged with macrophages when co-cultured, further suggesting a specific cell-cell interaction between HSCs and macrophages. Together these studies should advance our understanding of the role of macrophages within the HSC niche.

HHMI-41

The effect of early-life intestinal microbiota alteration on ileal, hepatic, and adipose DNA methylation in C57BL/6 mice

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Across many vertebrate species, antibiotic exposure in early life has been shown to increase adiposity. Prior studies from our lab have demonstrated that the intestinal microbiota perturbation induced by antibiotics, and not the antibiotics themselves, are sufficient to elicit this phenotype. This project aims to test the hypothesis that early-life intestinal microbiota disruption from antibiotic exposure results in changes in epigenetic methylation patterns in the ileal wall, liver and adipose of C57BL/6 mice. Pregnant mice were exposed to low-dose penicillin (LDP) or not (control), shortly before giving birth and through weaning. Their weights have been tracked since weaning, and serial DEXA scans have been performed since week of life (WOL) 11. These mice are now passing WOL 20 and the data are beginning to show differences between the experimental groups. Ileal, hepatic and adipose tissue was collected at WOL 4 and 8, and will be collected again at week 25. The DNA from these tissue samples has been isolated and an assay is currently being employed to assess methylation status on a genome-wide scale. Genetic regions of significant differences in methylation between cases and controls will prompt further investigation into the significance of these regions (i.e., gene body, promoter, transcription factor, etc.). We focus on the ileum because that is a substantial interface with gut microbiota (altered or normal) and host epithelial and immune cells, and on the liver and adipose tissue, since these are major nexuses for energy production and storage.

HHMI-42

Acute mechanical stabilization after injury attenuates heterotopic ossification

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Several factors are involved in physiologic repair after trauma. Disruption of any of these may result in aberrant healing. Heterotopic ossification (HO), the ectopic formation of bone in soft tissue, represents pathologic wound repair leading to chronic pain, diminished range-of-motion, and risk of infection and neurologic injury. One long-standing principle of wound management is tissue stabilization. Often performed using mechanical splinting, this minimizes strain through motion. The benefits of unloading on skin healing have been well documented, however, in regards to musculoskeletal trauma, it is unclear whether early- or delayed mobilization provides the best clinical result. Here we examined the relationship between immobilization and pathologic healing with a murine trauma/immobilization model of HO. Utilizing microCT imaging we demonstrated near absence of ectopic bone in mice that had been immobilized for 9-weeks versus controls (0.49±0.54 v. 7.25±2.58 mm^3). To isolate a therapeutic time-frame we performed delayed immobilization noting that sustained immobilization starting 3-weeks post-injury no longer decreased HO (14.78±7.04 mm^3). Given this timing, we examined the effect of immobilization on the pre-osseous or endochondral anlagen. Using pentachrome and SOX9 (cartilage marker) staining we demonstrated that no endochondral tissue was present at sites of injury after 3-weeks immobilization. Furthermore, while HO lesions are typically preceded by mesenchymal condensation as early as 1-week post-injury, we observed significant reduction in platelet derived growth factor receptor α ($PDGFR\alpha$)$^+$ and α smooth muscle actin (αSMA)$^+$ cells in immobilized tissue at both 1- and 3-weeks. This was supported by gross H&E demonstrating global reduction in cellular infiltrate 1-week post-injury. These findings suggest that acute immobilization in the first week after injury may be sufficient to attenuate HO formation.

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HHMI-43

Characterization of neurons involved in descending pain control reveals delta and mu opioid receptor expression

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The rostral ventromedial medulla (RVM) is a brain region that gates descending pain control by intercepting incoming sensory information entering the spinal cord. Three populations of medullospinal neurons (ON, OFF, and neutral cells) have been described based on their firing patterns during painful stimuli. These firing patterns are sensitive to exogenously administered opioids and homeostatic changes, which can augment or diminish pain thresholds. Intramedullary morphine injections are analgesic, purportedly due to direct μ-opioid receptor (MOR) mediated inhibition of ON cells and indirect activation of OFF cells. δ-opioid receptors (DOR) may also contribute to neural activity in this descending circuit either independently or cooperatively with MOR. However, the neurons that express these receptors have not been characterized, and thus the mechanism by which they regulate pain transmission in the spinal cord remains elusive. In this study, we used knock-in reporter mice that express DOReGFP and MORmCherry fusion proteins to reveal DOR- and MOR-expressing neurons in the RVM. Retrograde tracing and immunohistochemical experiments revealed that 46% of medullospinal neurons are DOR^+. Further characterization showed that 68% of MOR^+ and 78% of DOR^+ neurons are GABAergic while less than 1% of DOR^+ neurons are serotonergic, a hallmark neurotransmitter used by neutral cells. MOR and DOR expression also overlaps in the RVM, with 57% of DOR^+ neurons co-expressing both receptors. Additionally, we used chemogenetics to control GABAergic RVM neurons in freely moving mice via virally-delivered $hM_4D(G_i)$ and discovered that inhibition of these neurons is analgesic. Together, our results suggest that ON cells may not exclusively express MOR and that opioid mediated analgesia is orchestrated by disinhibition of the medullary input to the spinal cord. With ongoing functional studies, our results will elucidate how DOR and MOR cooperate to fine-tune descending pain control.

HHMI-44

Analysis of epithelial-stromal interactions and their relevance to lung cancer

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The communication that transpires between epithelial cells and their underlying stroma is an important but poorly understood aspect of organismal biology. If aberrantly regulated, these interactions can prove to be tumorigenic. It has been known for many years that cancer-associated fibroblasts (CAFs) promote and sustain the growth of tumors, but the mechanisms underlying their intercellular conversations have remained elusive. Previous work in our lab has identified a novel mechanism of communication in which CAFs secrete cardiotrophin-like cytokine factor 1 (CLCF1), a cytokine that binds the ciliary neurotrophic factor receptor (CNTFR) on tumor cells and promotes neoplastic growth. The CNTFR is a component of the tripartite receptor complex formed by CNTFR-gp130-LIFR and is capable of activating several oncogenic signaling cascades, including Jak-STAT. Using xenograft models, we have found that CLCF1 overexpression by CAFs increases tumor growth, whereas knockdown of CNTFR on lung tumor cells decreases overall growth. As a next step, we have generated a high-affinity CNTFR decoy that inhibits CLCF1-CNTFR signaling. I plan to use this novel reagent to elucidate the mechanism by which CNTFR activation alters intercellular signaling, leading to increased tumor cell growth. Through *in vivo* studies, I will also assess the efficacy of this CNTFR decoy as a form of cancer therapy. Moreover, I plan to use next-generation sequencing technologies to identify novel regulators of fibroblast-tumor cell communication. To this end, I will perform RNA-Seq on paired human normal lung fibroblasts (NLFs) and CAF cultures that I have isolated from patients with non-small cell lung cancer. By utilizing RNA-Seq to identify additional transcriptional differences between NLFs and their CAF counterparts, I hope to identify other novel mechanisms of CAF-cancer communication that will further advance our understanding of how fibroblasts communicate with tumor cells.

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HHMI-45

Exosomes from mesenchymal stem cells package anti-inflammatory cytokines to modulate immune function in allotransplantation

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Mesenchymal stem cells (MSCs) are immunomodulators investigated in allotransplantation, yet their mechanism of action is poorly defined. We hypothesized that MSCs package anti-inflammatory cytokines into exosomes (EXO) which execute the immunomodulatory effects of MSCs, and differential EXO packaging could be driven by oxygen tension and inflammatory milieu. Our aims were: (1) Determine whether MSCs secrete EXOs; (2) Characterize MSC-EXO cargo under differential conditions; (3) Investigate MSC-EXOs in preventing allograft rejection. Lewis rat-derived MSCs were cultured in normoxia (21% O_2), hypoxia (5% O_2), or with 100U IFNγ. MSC-EXOs were isolated by ultracentrifugation and confirmed by TEM, immunoblot, and flow cytometry for EXO markers. Transcriptional and translational MSC-EXO cytokine cargo were characterized by qRT-PCR and flow cytometry. Furthermore, MSC-EXOs were assessed for their capacity to induce Treg production and prevent acute rejection in an allogeneic rat hind limb transplantation model. MSC-EXOs from normoxic, hypoxic, and IFNγ-primed MSCs expressed EXO markers Cd63 and Cd81. Transcriptional MSC-EXO cargo included *iNOS* and *IDO*. Hypoxic MSC-EXOs packaged significant quantities of the anti-inflammatory cytokines IDO (11-fold, $p<0.001$), TGFβ (3.8-fold, $p<0.05$), iNOS (1.8-fold, $p<0.0001$), and IL-10 (1.5-fold, $p<0.05$) versus an isotype control. Normoxic MSC-EXOs induced a 6-fold increase in a $CD4^+FoxP3^+$ Treg population ($p<0.01$) versus a negative control; a 2-fold greater induction than normoxic MSCs ($p<0.0001$). In an allotransplantation model, DiI-labeled MSC-EXOs were taken up by perivascular cells, which may allow them to attenuate acute rejection episodes. In conclusion, MSC-EXOs package antiinflammatory mediators of MSC immunomodulation and stimulate Treg production. Preliminary allotransplantation studies suggest that MSC-EXOs localize to the perivascular space, and therefore may offer a novel approach in preventing rejection in allotransplantation.

HHMI-46

Effects of combined immune activation and peripheral radiation cancer therapy on brain function in a murine tumor model

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Cancer patients often report behavioral and cognitive changes following cancer treatment. These effects are also seen in patients who receive peripheral but no cranial irradiation. A proinflammatory environment in the brain may mediate these effects. Neuroinflammation mediates cognitive impairments in other neurological disorders. Its role in cancer-related neurological dysfunction is relevant given novel treatments combining radiotherapy (RT) and immune activation. These treatments demonstrate remarkable efficacy with respect to tumor outcomes by enhancing the proinflammatory environment in the tumor. However, little is known about how these treatments might affect the brain, in individuals with or without tumors. Here we test the hypothesis that immunotherapy combined with peripheral RT will have detrimental behavioral and cognitive effects as a result of an enhanced proinflammatory environment in the brain. C57BL/6J mice with or without injected hind flank Lewis Lung carcinoma were used for all experiments (n=10 mice per experimental group or 80 mice in total). Checkpoint inhibitor immunotherapy, using an anti-CTLA-4 antibody, and precision CT-guided peripheral RT alone and combined were used to closely model clinical treatment. Mice were given access to two identical objects and 24 hours later reintroduced to one familiar object and one novel object. Mice that recognize the familiar object will spend more time exploring the novel object. The percent time exploring the novel object out of the total time exploring both objects is calculated to determine object recognition. In the animals that did not receive tumors, object recognition was seen in control mice and mice receiving either RT or anti-CTLA-4 alone, but not in mice receiving both treatments. In mice receiving tumors, only the mice that received RT alone showed novel object recognition. Thus, while combining immune activation and RT optimizes tumor control, it is associated with cognitive impairments.

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HHMI-47

Developing a co-culture system to investigate the role of extraocular muscles in the growth and guidance of cranial nerves

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Individuals with congenital forms of paralytic strabismus, a disorder in which patients' eyes are misaligned due to functional extraocular muscle paralysis, often have extraocular muscle (EOM) co-contraction and other clinical evidence of aberrant innervation. Animal models of genetic forms of paralytic strabismus reveal both cranial nerve hypoplasia and EOM misinnervation, suggesting that EOMs release cues that direct axon guidance of the innervating nerves. We developed a co-culture system pairing murine embryonic oculomotor nuclei with developing extraocular muscles to study their interactions *in vitro*. Two reporter lines were used: a GFP reporter fused to an Islet-1 promoter labeled developing oculomotor nuclei and axons, while a TdTomato reporter line crossed to myf-5-cre mice labeled the developing extraocular muscles. Oculomotor nuclei and extraocular muscle anlages were dissected and cultured at embryonic day 11.5, then incubated for 14 days. Axonal growth and directionality were quantified, and media was collected for mass spectrometry analysis. In the presence of muscle explants, oculomotor axons exhibited clear directional growth towards the muscle anlage. Preliminary mass spectrometry identified several molecules secreted in co-cultures that were not present in control wells, including several proteins thought to be important in axonal guidance. We have successfully developed a co-culture system to probe the role of extraocular muscles in the growth and guidance of cranial nerves. Preliminary *in vitro* data supports our hypothesis that extraocular muscle directs the growth of oculomotor axons and releases extracellular matrix molecules that have been implicated in axon guidance. Future directions include investigating differences in the growth of oculomotor, trochlear, or abducens axons when cultured with their correct target muscles versus incorrect target muscles, evaluating the response of mutant explants versus wildtype explants, and using data from mass spectrometry to manipulate ligands and receptors in the system.

HHMI-48

Targeted inhibition of insulin-like growth factor 1 receptor signaling for potential treatment of human osteosarcoma

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Osteosarcoma (OS) is the most common primary human malignancy of bone. Increased IGF-1R signaling has been associated with progression and chemoresistance of OS. RNAi knockdown of *IGF1R* has been shown to increase the chemo- and radio-sensitivity of human OS (hOS). Thus, our lab is studying the novel use of IGF-1R dominant negative mutants as an effective adjuvant to conventional cytotoxic drugs.

Dominant negative mutants were constructed from the IGF-binding domain of the α subunit (sa-IGF1R), soluble β subunit (sb-IGF1R), and membrane-bound β subunit (mb-IGF1R) of IGF-1R, then cloned and packaged into recombinant adenoviruses. Expression of mutants by viral constructs was confirmed by Western blot and qPCR. High transduction efficiency was observed in 143B (metastatic hOS) cells. IGF-1-stimulated cell proliferation was inhibited by all mutants ($p<0.05$) on crystal violet assay. Decreased cell migration with observed with all mutants in a wound-healing assay. In quantitative cell cycle analysis by flow cytometry, all mutants reduced the percentage of cells entering S/G2 phases upon IGF-1/-2 stimulation. *In vivo*, all mutants inhibited subcutaneous and intramuscular tumor growth up to 2 weeks post-injection ($p<0.001$) in athymic nude mice as measured by bioluminescent imaging. Histological examination revealed significant necrosis and reduced proliferation among the mutant AdIGF1R-transduced 143B cells. Taken together, our results suggest that dominant-negative mutants of IGF-1R signaling hold promise as a potential therapeutic for hOS.

HHMI-49

Neutrophil hypochlorous acid contributes to cisplatin ototoxicity

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A common side effect of the chemotherapeutic drug Cisplatin is sensorineural hearing loss. We elucidate here the mechanism driving neutrophil mediated augmentation of cisplatin ototoxicity using a model of cochlear cells, HEI-OC1 cells. As a model mediator of neutrophils we selected hypochlorous acid (HA) as

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it showed cytotoxic reactivity against nucleic acids and proteins. Upon exposure to Cisplatin, HEI-OC1 cells up-regulated CXCL2; a chemokine that recruits neutrophils and monocytes to areas of inflammation. These immune cells, via the action of NOX2 and Myeloperoxidase (MPO), generate the reactive oxygen species HA and Hydrogen Peroxide (H202). In our MTT assays, we showed that sodium hypochlorite, a sodium salt of HA, reduces HEI-OC1 cell viability in a dose-dependent manner. Co-treatment of MPO and H202 was found to be cytotoxic to HEI-OC1 cells and additionally appeared to augment cisplatin-mediated cytotoxicity of HEI-OC1 cells. In addition, Cisplatin treatment upregulated TLR4 expression in HEI-OC1 cells. HMGB1, a model damage-associated molecular pattern (DAMP) that is released during necroptotic cell death and binds to TLR4, was found to upregulate CXCL2 expression in HEI-OC1 cells and NOX2 expression in HL60 cells (a model of neutrophils). Altogether, our findings suggest that cochlear inflammation is potentially involved in cisplatin ototoxicity through HA-mediated potentiation of cisplatin cytotoxicity to auditory sensory cells. Further studies are needed to show the involvement of neutrophils in cisplatin ototoxicity *in vivo* in addition to cisplatin-induced release of DAMP from auditory sensory cells.

HHMI-50

Constitutive androstane receptor – linking host nuclear receptor activity with changes in the gut microbiota

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The constitutive androstane receptor (CAR) is a critical nuclear receptor which plays a role in the regulation of xenobiotic and lipid metabolism, improved insulin sensitivity and reduction of body weight in mice. Regulation of CAR by gut microbiota has been implicated in studies showing higher CAR and target gene expression in germ-free versus conventional mice. Therefore, understanding the unique relationship between CAR and the microbiome would allow for significant therapeutic advancement in metabolic disease. Consequently, we conducted an experiment with male mice fed high fat milk fat (MF) diet for 7 weeks followed by 5 weekly treatments of CAR agonist 1,4-bis[2-(3,5-dichloropyridyloxy)] benzene (TCPOBOP). Control mice were fed MF diets and injected with corn oil vehicle control. TCPOBOP-treated mice showed significant weight loss, increased glucose tolerance, and altered expression of enzymes that regulate bile acids. Subsequently, male wild type (WT) mice and $CAR^{-/-}$ mice were maintained on regular chow diet, bedding was mixed twice per week, until 10 weeks of age. The $CAR^{-/-}$ mice displayed a higher rate of weight gain. Analysis of 16S rRNA microbial sequencing data from cecal contents displayed an increase in Clostridia and decrease in Bacteroidia class when compared to WT. In summary, activation with TCPOBOP causes metabolic changes while loss of CAR function results in distinct alterations of the gut microbiota. Understanding mechanisms behind this interaction will lead to advancements in targeting microbiota as a therapeutic option for the treatment of metabolic diseases.

HHMI-51

Identifying small molecule compounds that enhance the host innate immune response to infection

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Thrombospondin-1 (TSP1) is a calcium-binding, matricellular glycoprotein released during inflammation by platelets, endothelial cells, and myeloid-derived cells. A discrete region within the type 3 repeats (T3R) domain of TSP1 can restrict microbial killing of *Klebsiella pneumoniae (Kp)* by competitively inhibiting neutrophil elastase (NE) activity. A small molecule library screen of ~500,000 compounds was conducted to identify candidates that block the interaction involving TSP1 T3R domain and NE. Six high-ranking compounds were identified based on size, safety, and hydrophobicity. Utilizing the NE substrate N-(methoxysuccinyl)-Ala-Ala-Pro-Val-p-nitroanilide (AAPV-pna), we created a high-throughput assay to screen the identified compounds. Five of the six compounds effectively antagonized recombinant monomeric human TSP1 and enhanced NE hydrolysis of AAPV-pna, with the lead compound BC-1401 showing an IC_{50} ~22 μM. BC-1401 enhanced NE activity by dose-dependently inhibiting TSP1 under varying pH conditions, ionic strength, and in the presence or absence of calcium ions. BC-1401 itself had no effect on hydrolysis of AAPV-pna, indicating that the compound's ability to enhance NE activity was due to alterations in NE/TSP1 interactions. As prior cross-transfer serum experiments suggest that the major source of TSP1 regulating neutrophil microbial killing is contained within serum, we sought to determine whether platelet TSP1 cooperates in limiting neutrophil protease activity and microbial killing. We challenged thrombocytopenic mice deficient in the thrombopoietin receptor c-Mpl (*Mpl*$^{-/-}$) to *Kp*. Consistent with findings in *thbs1*$^{-/-}$ mice, *Mpl*$^{-/-}$ mice showed reduced bacterial dissemination to the spleen, liver, and blood following *Kp* intrapulmonary infection. Our findings suggest small molecule compounds that block TSP1/NE interaction enhance NE activity and implicate platelets in restraining neutrophil activation during bacterial infection.

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HHMI-52

Arbiters of cellular proteostasis: an exploration of the roles of the p97 segregase and the ubiquitin listerin ligase in the ribosome quality control pathway

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Heightened protein synthesis rates in cancer cells make them increasingly dependent on intact mechanisms of proteostasis: a cell's ability to regulate the expression, folding and turnover of the proteome. The Ubiquitin-Proteasome System (UPS) serves as a principal mediator of proteostasis, and consists of a cascade of conjugating enzymes that culminate in the attachment of ubiquitin (Ub) chains to proteins fated for proteasomal degradation. Recent investigations have focused on UPS protein quality control (PQC) pathways that exploit cancer's vulnerability to proteostatic disruptions as a means to inducing irresolvable, lethal proteotoxic burden. In this study we investigated a particular component of the UPS, the p97 segregase: an ATPase implicated in ribosome quality control (RQC), a UPS process by which tRNA-linked ubiquitinated polypeptides are released from stalled ribosomal complexes and shuttled for degradation. Employing allosteric and competitive inhibitors of p97 in colorectal carcinoma cells, we observed a several fold increase in Ub conjugate accumulation on polysomes when compared to controls, indicating a backlogging in the p97-mediated resolution of RQC substrates. To determine if the Ub conjugates were derived from aberrant nascent polypeptides, biotin-conjugated puromycin was utilized to label nascent chains from polysomes. However, upon Ub conjugate enrichment no difference was observed in biotin labeling of polysomes from p97-inhibited cells versus untreated cells, an observation which suggests that most Ub conjugates are derived not from actively translating polysomes, but from dissociated 60S subunits. To further explore this model, we resolved cytoplasmic fractions of p97 inhibitor-treated and untreated cells via centrifugation, and immunoblotted for Ub. These results indeed confirmed a several fold increase in Ub conjugate accumulation in the 60S fractions of p97 inhibitor-treated cells compared to controls. This observation has prompted inquiry as to the effect of p97 inhibition in the context of Listerin depletion, a RING ligase that binds 60S and facilitates Ub conjugation of tRNA-linked aberrant peptides. Future work will therefore involve knocking down Listerin to determine if the ligase acts upstream of p97 in the RQC model. Considering the importance of Listerin in PQC, it would be worthwhile to probe the capacity of this UPS effector as a druggable target in tumor cells. At the very least, characterizing the significance of p97 and Listerin on the proteomic landscape will help to further elucidate the mechanistic basis of protein triaging at the ribosomal level.

HHMI-53

BBS1 mutant mice have impaired fear conditioning, which is partially rescued by lithium treatment

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Bardet–Biedl syndrome (BBS) is a pleiotropic, heterogeneous, autosomal recessive ciliopathy. BBS phenotypes including obesity, retinopathy, hydrocephalus, and hypogonadism have been studied in mouse models. BBS patients are known to have an increased risk of psychiatric disorders; however, behavior has not been thoroughly studied in BBS mice. We performed behavioral tests on a homozygous knockin mouse model ($BBS1^{M390R/M390R}$) of the most common BBS mutation ($BBS1^{M390R}$) in humans. We discovered a novel behavioral dysfunction. Specifically, $BBS1^{M390R/M390R}$ mice have impaired cue and context fear conditioning (FC) [$p<0.01$]. To rule out confounding variables on the FC test, we performed control studies, which indicate that $BBS1^{M390R/M390R}$ mice have normal motor function including normal cerebellar function (rotarod performance test) and normal hearing (auditory brainstem response) compared to control mice. In addition, the CO_2 evoked freezing test revealed that $BBS1^{M390R/M390R}$ mice freeze similarly to control mice indicating that $BBS1^{M390R/M390R}$ mice have normal CO_2-induced fear function. These findings suggest that $BBS1^{M390R/M390R}$ mice show a specific behavioral deficit in fear conditioning. Interestingly, we have shown that lithium treatment rescues cue but not context dependent fear conditioning deficits. We are currently searching for the mechanism of the FC defect by investigating the hippocampus and amygdala. Field recordings show that long term potentiation of the hippocampus is not different between $BBS1^{M390R/M390R}$ mice and controls. Whole-cell patch recordings of pyramidal neurons of the lateral amygdala show that the frequency and amplitude of miniature excitatory postsynaptic currents (mEPSCs) are smaller in $BBS1^{M390R/M390R}$ mice compared to controls [$p<0.01$]. To test which brain regions account for the FC impairment, we are currently knocking out BBS genes in specific brain regions including amygdala using AAV-cre stereotaxic injections.

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HHMI-54

Disruptions in abducens nerve development can cause Duane retraction syndrome

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Duane retraction syndrome (DRS) is characterized by an absent or hypoplastic abducens nerve and aberrant innervation of the lateral rectus muscle by the oculomotor nerve. We developed a new mouse model of DRS to further elucidate its developmental etiology. We provide the first evidence supporting the hypothesis that any insult preventing the abducens nerve from innervating the lateral rectus in development can cause DRS. *Mafb*$^{WT/flox}$ mice were crossed to the ubiquitously expressing EIIa-cre to generate *Mafb*$^{WT/KO}$ mice used for heterozygous crosses, as *Mafb*$^{KO/KO}$ mice die at birth. *Isl1*^{MN}*:GFP* reporter mice were crossed to Mafb mice to visualize developing motor axons. Whole mount preparations of embryos at E11.5 were stained with anti-neurofilament, cleared in BABB and imaged by confocal microscopy. Orbits of embryos at E16.5 were dissected, stained with anti-actin α-smooth muscle, cleared in glycerol and imaged by confocal microscopy. *Mafb*$^{KO/KO}$ mice have loss of rhombomeres 5 and 6 in development, absent abducens nerves and nuclei, and fusion of the glossopharyngeal nerves with the vagus nerves. *Mafb*$^{WT/KO}$ mice have hypoplastic abducens nuclei and nerves. In the orbits of both *Mafb*$^{KO/KO}$ and *Mafb*$^{WT/KO}$ mice, the oculomotor nerves aberrantly branch to innervate the lateral recti, recapitulating human DRS. *Mafb* expression is restricted to the hindbrain in development and therefore *Mafb* is not expressed in developing oculomotor axons. We therefore establish *Mafb* mice as a model for DRS, and demonstrate that specifically disrupting abducens nerve development can cause DRS by allowing the oculomotor nerve to aberrantly innervate the lateral rectus. We provide the first evidence to support the hypothesis that insults to the developing abducens nerve *in utero* can cause DRS.

HHMI-55

Genome-wide CRISPR/Cas9-based screens for host factors required for *Vibrio parahaemolyticus* type 3 secretion system-mediated cytotoxicity

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Type 3 secretion systems (T3SSs), needle-like nanostructures evolved to inject bacterial effector proteins directly into the eukaryotic cytosol, are widespread virulence factors among pathogenic Gram-negative bacteria. *Vibrio parahaemolyticus*, a common cause of seafood-borne enteritis, encodes two T3SSs (T3SS-1 and T3SS-2). Only T3SS-2 is critical for the pathogen to colonize and cause disease in the host intestine, but both T3SS-1 and T3SS-2 cause cytotoxicity in cultured host cells via distinct mechanisms. Taking advantage of recently established CRISPR/Cas9 technology, we developed a positive selection screen to identify host genes required for T3SS-1- and T3SS-2-mediated cytotoxicity. A complex library of guide RNAs targeting each human gene was transduced into a Cas-9 positive intestinal cell line and by infecting with *V. parahaemolyticus* expressing either T3SS-1 or T3SS-2, the population was enriched for those perturbations that conferred resistance to infection. The compelling results from these two selections implicated several coherent and distinct pathways to resistance. We discovered that host-cell surface sulfation and fucosylation are key pathways targeted by *V. parahaemolyticus* to engage cells. Sulfation proved to promote bacterial adhesion and thereby facilitated subsequent T3SS-1 killing, whereas fucosylation was critical for T3SS-2 activity, promoting effector translocation into host cells by facilitating the insertion of the T3SS-2 translocon into the host cell membrane. Overall, our findings illustrate that CRISPR/Cas9-based genome-wide screens will enable fundamental new understanding of the host response pathways that underlie T3SS-mediated virulence.

HHMI-56

A CRISPR/Cas9 based genome-wide knockout screen identifies novel host dependency factors for HIV infection

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HIV relies upon host proteins to support its infection. To identify these HIV dependency factors (HDFs), we performed a genome-scale forward genetic screen in the CD4$^+$ T cell line CCRF-CEM. We delivered ~190,000 single-guide RNAs (sgRNAs) into CCRF-CEM cells stably expressing Cas9 and a GFP reporter driven by HIV LTR. Following HIV infection, we isolated viable, un-infected cells. By massively parallel sequencing, we then identified highly

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enriched sgRNAs corresponding to five candidate HDFs: CD4, *CCR5*, *TPST2*, *SLC35B2*, and *ALCAM*. Cells lacking TPST2 or SLC35B2 were strongly protected against HIV infection (3% death for TPST2 KO, 1% for SLC35B2 KO) compared to control cells expressing non-targeting sgRNAs (68%). Add-back of the gene ablated this protection (85% death for TPST2, 68% for SLC35B2). TPST2 mediates tyrosine sulfation of CCR5 in the Golgi, which is known to facilitate HIV entry. SLC35B2 is a cytosol-to-Golgi transporter for PAPS, the universal donor for protein sulfation. SLC35B2 KO thus likely confers protection by depriving TPST2 of its sulfate source. As expected, loss of either gene blocked HIV entry (4% for *TPST2*, 1% for *SLC35B2*, 40% for control), while gene add-back reversed the block (69% for *TPST2*, 33% for *SLC35B2*). Loss of ALCAM completely disrupted the ability of CCRF-CEM cells to form clumps under normal culture conditions. Furthermore, ALCAM-null cells failed to aggregate with wild-type cells, suggesting that loss of ALCAM may confer protection against cell-to-cell HIV transmission. In agreement with this hypothesis, we found that ALCAM-null cells are strongly protected from HIV infection compared to control cells upon co-culture with infected control cells (13% death vs 67% control) and that this protection is ablated upon add-back of the gene (87%). Our findings identify potential host-directed therapeutic targets for HIV and illustrate the potential of genome-scale genetic knockout screens in discovering novel dependencies of human pathogens.

HHMI-57

Stromal cell-derived factor-1α gene therapy accelerates diabetic wound healing

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Chronic wounds disproportionately affect individuals with diabetes, markedly impacting quality of life and lifespan. Defective neovascularization in ischemic diabetic tissue is a major contributor to delayed wound healing. Studies have shown that stromal cell-derived factor-1α (SDF-1), a chemokine that plays a critical role in initiating neovascularization through recruitment of circulating progenitor cells (CPCs) to ischemic tissue via a HIF-1α dependent mechanism, is diminished in diabetic wounds. Using a non-viral minicircle DNA vector, we study the effect of restoring SDF-1 expression in diabetic wounds.

A minicircle vector expressing SDF-1 and luciferase (SDF1-Luc MC) was constructed. Humanized excisional wounds were created on the dorsum of wild type and diabetic mice. Wounds were treated with SDF1-Luc MC and compared to untreated controls. Wounds were photographed and assessed with bioluminescent imaging every other day. Systemic levels of SDF-1 were monitored. Tissue was harvested for histology and qRT-PCR. Bioluminescent imaging demonstrated successful uptake and sustained expression of naked minicircle injected into the dermal layer of wounds. The SDF1-Luc MC treated diabetic group demonstrated accelerated wound healing compared to untreated diabetic controls. Localized treatment with SDF1-Luc MC did not produce supraphysiologic levels of SDF-1 expression in diabetic mice relative to wild type controls. Expression of SDF-1 in ischemic tissue is crucial to the process of healthy wound healing. The accelerated wound healing observed in diabetic wounds treated with SDF1-LUC MC demonstrates the therapeutic potential of SDF-1 augmentation.

HHMI-58

Laminar visual area V4 response during covert and overt attention

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The functional architecture of the cerebral cortex is characterized by distinct lamination patterns, defined in part by cell type, connectivity and response properties. Information is processed within a cortical column and then sent either forward to hierarchically more advanced cortical areas, or as feedback to areas synaptically closer to feedforward input. The frontal eye field (FEF), located in prefrontal cortex, is a major source of attention-related, modulatory feedback to visual areas, such as V4. Anatomically, the FEF projects to all layers of V4, whereas other feedback sources have more specific laminar targets. Previous studies of attentional modulation of neural activity in V4 have found laminar differences in local field potentials, but not in spiking activity. We recorded from area V4 in two macaques using a linear microelectrode array during a task that separated overt attention (preparation and execution of orienting eye movements) from covert attention (attention without overt orienting). Using multiclass support vector machines, we were able to accurately distinguish on a trial-by-trial basis whether a neural population's receptive field was the site of overt attention, covert attention, or was orthogonal to attention. We found additional differences in burst activity and angular tuning across conditions. While superficial neurons had a higher median firing rate, and were more tuned to stimulus orientation, the distribution of neurons significantly modulated by attention was not influenced by laminar depth. Our results demonstrate that covert and overt attention can be distinguished on a neuronal population level in V4. These results are consistent with the notion that the FEF drives attentional modulation in V4, and does so across all layers.

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HHMI-59

Assessment of cell types that secrete semaphorin 3F (Sema3F) to control dendritic spine distribution and morphogenesis in the postnatal CNS

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The secreted semaphorin Sema3F is a negative regulator of dendritic spine development and synaptic structure – functions that are accomplished through association with its holoreceptor components neuropilin-2 (Npn-2) and plexin A3 (PlexA3). Indeed, mice with null mutations in genes encoding Sema3F, Npn-2, or PlexA3 exhibit increased spine number and altered spine morphology, along with aberrant spine distribution in dentate gyrus (DG) granule cells (GC) and cortical layer V pyramidal neurons. Sema3F-mediated loss of spines is thought to be restricted by the specific localization of Npn-2 receptors (for example, in cortical pyramidal neurons along the primary apical dendrite). The *in vivo* source of Sema3F responsible for these effects remains unknown. Through analysis of postnatal mice with myc-tagged Sema3F expressed from its endogenous locus, we determined that Sema3F is diffusely localized in the olfactory bulb, inferior colliculus, and cerebral cortex. Preliminary *in vitro* evidence shows that cortical pyramidal neurons derived from E14.5 are often surrounded by non-pyramidal neurons possessing a stronger Sema3F signal. It is possible that these non-pyramidal neurons are inhibitory interneurons that secrete Sema3F which subsequently acts on excitatory pyramidal neurons. To test this idea, we are utilizing Cre drivers in mice to disrupt secretion of Sema3F from all, or specific classes, of interneurons including those positive for somatostatin, vasoactive intestinal peptide, and parvalbumin. However, pyramidal neurons will also be assessed for potential secretion of Sema3F by utilizing a pan-excitatory Cre driver. We will sparsely label pyramidal neurons through striatal injections with glycoprotein-deleted rabies virus encoding GFP to visualize spine morphology. We will then assess spine morphology and number in DG GCs and layer V pyramidal neurons in sections of P28 mouse cortex.

HHMI-60

Modeling glioblastoma through cancer stem cell organoids

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Glioblastoma (GBM) is a universally lethal cancer for which therapeutic options are limited. GBM tumors display complex cellular hierarchies driven by cancer stem cells, which rely on complex interactions with the microenvironment. Traditional culture models fail to recapitulate the cellular heterogeneity, three-dimensional (3D) tumor architecture, and microenvironmental gradients of parental tumors. Here, we utilized a 3D organoid culture system derived directly from GBM specimens to develop a GBM model that effectively maintains microenvironmental heterogeneity and contains spatially defined gradients of stem and non-stem cells. Once formed, tumor organoids grow up to several millimeters in size and display regional heterogeneity with a rapidly proliferating rim of $SOX2^+$, $OLIG2^+$, and TLX^+ cells surrounding a quiescent, hypoxic core with low cellularity. GBM organoids thus reflect the varied tumor microenvironment and permit simultaneous culture of diverse populations of stem and non-stem tumor cells. Intracranial xenografts from patient-derived organoids form orthotopic tumors with greater histologic similarity to the parental tumor versus standard sphere culture, including increased invasion and differential radiosensitivity. Our data support a cooperative model of tumor cell interactions wherein the proliferative capacity largely arises from cells in high oxygen, high nutrient microenvironments, while the hypoxic core retains a broader range of long lived cells of varying states within the cellular hierarchy. This model will be utilized to recapitulate particular aspects of tumor niche biology *in vitro* that are not achievable by standard 2D culture. Specifically, we will compare cells of the organoid rim and core to assess the role of hypoxia in promoting stem-like characteristics as well as the ability of cells to interconvert between microenvironments. Further elucidating this role of the microenvironment in regulating tumor cell function and identity may inform future therapeutic approaches to targeting treatment-resistant GBM stem cell populations.

HHMI-61

SRSF5 and other splicing factors in radiation response

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Radiation therapy is one of the most common cancer treatment modalities. Despite its ubiquitous use, radiation is considered to be a "blunt" technology, as it harms indiscriminately both cancer and normal cells. To better understand how human cells respond to radiation stress, we examined alternative splicing, a component of the gene expression response to radiation that is largely unexplored. Specifically, we studied the expression of splicing factors, trans-acting RNA-binding proteins that are essential for alternative splicing. To accomplish this, we exposed cultured B-cells from 10 individuals to 10 Gy of ionizing radiation, and performed RNA sequencing before, and two and six hours following radiation treatment. From about 60 million reads per sample, we determine the expression levels of over 60 splicing factors, and quantified eight types of

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splicing events, such as cassette exons and retained introns. We found that the gene expression levels of 26 splicing factors, including *HNRNPH1*, *PTBP1*, *SRSF5*, and *SRSF6*, changed significantly (ANOVA, FDR<5%) in irradiated cells. In addition, we discovered that radiation-induced alternative splicing of these proteins modulates their expression. For example, in *SRSF5* radiation stress results in the exclusion of an exon that contains a premature termination codon, which thereby prevents nonsense-mediated decay and contributes to the increase of its expression level. This led us to survey more broadly the splicing patterns in irradiated cells. We found that of the over 20,000 splicing events detected, ~800 were radiation responsive. These radiation-induced splicing events occurred primarily in genes involved in RNA processing, DNA damage response, and cell cycle regulation. The splicing factors that mediate these events are potential targets for modulating cellular radiation sensitivity. In this presentation, we will describe our findings of splicing factors and alternative splicing events as components of cellular response to ionizing radiation.

HHMI-62

Development and validation of a novel isocitrate dehydrogenase 1-mutant astrocyte cell line as a model for high-grade gliomas

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High-grade gliomas (HGGs) are devastating malignancies of the central nervous system, and few treatment options are available. In the most malignant form of the disease, over 90% of patients will succumb to their tumor within 5 years after standard of care treatment. It is now clear that gliomas are molecularly heterogeneous entities, with various mutations defining many distinct sub-types with important therapy implications. However, almost all HGGs are treated with a limited array of therapies. Isocitrate dehydrogenase 1 (IDH1), a gene recently found to be mutated in many gliomas, is involved in the conversion of isocitrate to 2-oxoglutarate. The IDH1 R132H mutant enzyme converts 2-oxoglutarate to the oncometabolite (R)-2-hydroxyglutarate (D2HG), which leads to profound metabolic alterations in tumor cells. Recent studies indicate that mutations in IDH1 may also induce altered double-stranded break repair, differential sensitivities to chemo-radiotherapy, and changes in chromatin modifications. Here, we present the creation of a novel astrocyte cell line harboring an engineered heterozygous IDH1 R132H mutation at the endogenous gene locus. We confirmed expression of the engineered mutation at the protein level, and we have characterized this cell line in a comprehensive panel of functional assays. We demonstrated that our mutant cell clones secrete high levels of D2HG, and confirmed that the levels of this oncometabolite can be suppressed with small molecule inhibitors of mutant IDH1. We also characterized the DNA damage response network in IDH1-mutant cells using high-content DNA damage foci assays and in clonogenic survival assays. To our knowledge, this is the first report of an astrocyte cell line harboring an engineered, heterozygous R132H mutation at the endogenous locus. This novel cell line represents a new model system for studying gliomas and has tremendous applications for further cell characterization, mechanistic studies, and drug screening.

HHMI-63

Unraveling the controversy of bisphosphonates as vascular calcification therapy using a nanoanalytical approach

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Vascular calcification significantly predicts atherosclerotic plaque rupture and acute cardiovascular events. Bisphosphonates have been proposed as a therapy to treat vascular calcification. However, retrospective studies of women taking bisphosphonates paradoxically indicated increased risk in patients with prior acute events. We recently demonstrated that calcifying extracellular vesicles (EVs) released by vascular smooth muscle cells within the plaque aggregate and nucleate calcific mineral. We hypothesize that bisphosphonates block EV aggregation and arrest existing mineral growth, freezing calcifications in a high-risk morphology that hastens plaque rupture. EV-mediated vascular calcification and the potential to pharmacologically impede this process remain poorly understood. This study visualized for the first time EV aggregation and calcification at single-vesicle (100 nm) resolution, via scanning electron microscopy. Three-dimensional (3-D) collagen hydrogels incubated with calcifying EVs modeled fibrous cap calcification, serving as an *in vitro* platform to image EV-driven mineral formation and test candidate drugs for the potential to inhibit or reverse vascular calcification. EVs aggregated either along or between collagen fibrils. Energy-dispersive x-ray spectroscopy analysis confirmed that EV aggregates contained calcium and phosphorous, the building blocks of calcific mineral. The addition of the bisphosphonate ibandronate resulted in a decrease in the number ($p=0.023$, $n=3$ per group, 9 fields per sample), size, and morphology of calcific EV aggregates. These findings agree with our hypothesis that bisphosphonates alter EV-driven calcification. Further, our analyses confirmed that our 3-D collagen hydrogel system, mimicking the atherosclerotic cap, is a viable platform to study EV-mediated mineral nucleation and evaluate potential therapeutic strategies for cardiovascular calcification.

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HHMI-PDF-64

Characterization of the sirt6 knockout *Drosophila melanogaster*

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It is known that midbrain dopaminergic neurons demonstrate differential vulnerability to Parkinson's disease (PD) pathology and aging. Here, we took a novel approach to elucidate the molecular distinctions that render groups of midbrain neurons resistant to PD-induced neurodegeneration. Specifically, we carried out a meta-analysis of published human-derived microarray datasets to identify the genes that show a similar expression signature in populations of resistant neurons, such as the medial substantia nigra and the ventral tegmental area (VTA) neurons, and an altered signature in vulnerable neurons, such as those of the lateral substantia nigra. We identified *sirt6*, which codes for a component of the DNA repair complex, as a candidate neuroprotective gene as it has high levels of expression in the VTA and the medial substantia nigra and low expression levels in the lateral substantia nigra. We used immunohistochemical analysis to confirm this differential expression pattern of *sirt6* in the midbrain of wild type mice. In order to further investigate the effects of altered *sirt6* expression on the nervous system, we used CRISPR genome editing tools to create a *sirt6* knockout *Drosophila melanogaster* model. Our preliminary histological analysis reveals that the recessive mutation leads to early-onset neurodegeneration, as evidenced by a doubling of neuropil vacuolization in 30-day-old flies. We plan to further investigate the histological and behavioral phenotypes of this recessive mutation by assessing DNA damage accumulation in the brain, as measured by pH2Av protein levels, and evaluating the flies' climbing activity in a standard climbing test, respectively. We discuss the potential usefulness of our findings with regards to gaining a deeper understanding of differential vulnerability and DNA damage repair in the setting of the aging brain and Parkinson's disease pathology.

HHMI-65

Long-term analysis of the function and composition of the damaged, adult mouse utricle

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The utricle requires sensory hair cells to detect linear acceleration such as gravity. Previous studies suggest that the postnatal mouse utricle contains cells with the innate ability to regenerate new hair cells after damage. Using a previously established hair cell damage model with 3,3′-iminodipropionitrile (IDPN) as a synthetic vestibulotoxic nitrile compound, we have compared and correlated the histological changes of the adult mouse utricle to recovery of vestibular function as measured by vestibular evoked potentials (VsEPs) one week to six months post damage.

One week after injection, >95% of IDPN-treated mice showed elevated VsEP thresholds in comparison to saline-treated controls. One, three, and six months after damage, 88.2%, 60.0%, and 66.7% of treated mice showed elevated thresholds, respectively. Treated mice had significantly higher thresholds at one week ($p<0.01$) but not at later time points examined. As a group, IDPN-treated mice had significantly longer P1 latencies and lower P1-N1 amplitudes than those of saline-treated controls. $Plp1^{CreERT/+};R26R^{tdTomato/+}$ mice injected with IDPN had significant hair cell loss in the utricular sensory epithelium in both the striola and extrastriola (28.6% and 27.5% remaining, respectively). Six months after injection, hair cell numbers increased to 51.4% and 56.2% relative to controls in the striola and extrastriola, respectively. The number of traced hair cells also increased from one week to six months post damage. After IDPN-induced loss of hair cells and utricular function, a partial recovery of both is observed. Ongoing studies aim to better characterize the source and functionality of the newly generated hair cells.

HHMI-66

$CD8^+$ T cell responses in viremic controllers as a model for durable control of HIV infection

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HIV-1-specific $CD8^+$ T cells appear in concert with a decline in peak viral load in acute infection, well before neutralizing antibodies are produced, and are thought to contribute to relative control of HIV as a quasi set point is achieved. Though a role for these cells in viral control is well established, there is marked heterogeneity in the antiviral effectiveness of these responses, as measured by targeting of specific viral proteins, proliferative capacity, expression of negative immunoregulatory molecules, T cell receptor (TCR) clonotypes, and escape mutations. In order to define the properties of $CD8^+$ T cells associated with durable control, we are performing a detailed analysis of these responses in viremic controllers. These individuals, defined by a detectable plasma viral load of < 2000 RNA copies/ml in the absence of therapy, are of interest as they model a functional cure of HIV infection. Longitudinal samples of peripheral blood mononuclear cells from viremic controllers are being evaluated regarding the epitope-specificity of responses by IFN-γ Elispot assay, persistence by T cell receptor analysis, and antiviral efficacy by *in vitro* elimination assays. In addition, mechanisms of control are being addressed through studying the transcriptional profile and TCR clonotypes of proliferative and

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non-proliferative epitope-specific CD8+ T cell populations via RNA-seq analysis. Preliminary data thus far suggest the breadth of epitope-specific responses in viremic controllers is largely stable over time. Additionally, viremic controllers appear to have a larger breadth of expandable low frequency responses in peripheral blood compared to chronic progressors. Better understanding the phenotypic, functional, and transcriptional signatures of effective HIV-specific CD8+ T cells in viremic controllers may help in the development of preventive and therapeutic HIV vaccines, and will be important for HIV cure strategies where these cells will be needed to clear virus infected cells.

HHMI-67

The dependency of β-cell regeneration on hyperglycemia: are high glucose levels required to generate new β-cells *in vivo*?

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Human β-cell failure leads to diabetes and lifelong insulin therapy due to limited regeneration of β-cells. In contrast, β-cell ablation in zebrafish leads to regeneration. Studying this capability may identify therapeutic targets. While blood glucose is known to affect β-cell health, its role in regeneration is unclear. Preliminary data indicate blunted regeneration in zebrafish after insulin treatment, suggesting either lowered glucose, or insulin, inhibits regeneration.

This research tests if β-cell regeneration in zebrafish is altered by normoglycemia. To lower glucose independent of insulin action we used a sodium glucose co-transporter 2 inhibitor (SGLT2i) to increase urine glucose excretion. The subsequent effect on β-cell regeneration is being evaluated via β-cell fluorescence, blood glucose measurement, and islet immunohistochemistry (IHC).

SGLT2i treatment lowered blood glucose in both non-regenerating and regenerating zebrafish as did insulin. While blood glucose returned to baseline in regenerating controls, SGLT2i-treated fish remained hyperglycemic. IHC showed fewer insulin positive cells in SGLT2i-treated fish than in controls.

These results show SGLT2i treatment in zebrafish reduces blood glucose; thus normoglycemia may lead to insulin-independent inhibition of β-cell regeneration, indicating hyperglycemia is a key factor. However, recent studies show SGLT2is may protect islets by reducing β-cell glucotoxicity. Increased glucagon release from islet α-cells may also confound results so we must evaluate the role of islets in a regenerating model. Further study will confirm above findings and investigate glucose-sensitive mechanisms underlying regeneration.

HHMI-68

Optogenetic activation of central auditory pathways mediated by systemic gene delivery to the cochlear nucleus

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Auditory brainstem implants (ABI) can improve hearing in deaf patients who are not candidates for cochlear implants (CI) due to anatomic considerations. Overall, ABI performance is modest compared to the CI. An optically based ABI may improve outcomes by reducing non-specific activation of non-auditory neurons of the cochlear nucleus (CN) and enhancing channel selectivity. Here, we assess systemic gene transfer of opsins to the CN *in vivo* to enable the next step in translation of optogenetic technology for ABIs.

Adeno-associated viral vector (AAV) serotype 2/9 carrying a synapsin or CAG promoter for the Chronos-GFP coupled gene were injected into the superficial temporal vein or tail vein of C57BL/6J or CBA mice. After a minimum two week incubation period, craniotomies were performed to expose the dorsal CN (DCN) and inferior colliculus (IC). Pulsed collimated blue laser light was delivered to the surface of the DCN and a recording probe was placed in the IC along the tonotopic axis. After testing, brains were stained for GFP expression in the CN.

Histology of both tail vein and temporal vein injected mice demonstrated expression of Chronos-GFP in the CN. Preliminary data from a temporal vein injected mouse demonstrated neuronal firing in the IC in response to optical stimulation. As the laser was moved across the tonotopic axis of the DCN, there was a shift in location of activation within the IC, correlating to the spatially specific projections from the CN to the IC. Tail vein injected mice were not optically responsive.

This study is the first to demonstrate 1) gene transfer of opsins to the central auditory pathways via systemic delivery and 2) light-evoked multi-unit activity in the cochlear nucleus. Further studies will clarify whether increased temporal resolution of light stimuli can be achieved. Non-invasive approaches of opsin delivery to auditory neurons moves an optically-based ABI closer to clinical translation.

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HHMI-69

Smad4 regulation of colonic epithelial cell homeostasis

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Smad4 is a central mediator of the TGF-β/BMP signaling pathway. Germline mutations in *Smad4* are associated with juvenile polyposis syndrome and inactivating somatic *Smad4* mutations are observed in approximately 20% of colorectal carcinomas and are associated with poor prognosis. In order to understand the role of Smad4 in colonic epithelial homeostasis, we generated tissue-specific conditional (tamoxifen-inducible) *Smad4* knockout mice ($CK19^{CreERT}$; $Smad4^{fl/fl}$) in which approximately 20% of colonic crypts lost Smad4 expression. Smad4 null crypts exhibited an expanded proliferative zone, but did not yield intestinal tumors. However, in the background of Apc^{D1638} mutation Smad4 conditional null mice exhibited increased (10-fold) tumorigenesis. We have subsequently generated $Lrig1^{CreERT}$;$Smad4^{fl/fl}$ mice that exhibit loss of Smad4 in 80% of colonic glands. RNAseq comparison of Smad4 WT and Smad4 null mouse colonic epithelium coupled with pathway analysis suggested increased MAP Kinase signaling associated in the Smad4-deficient colonic epithelium. We confirmed that the Smad4 deficient colonic glands exhibited increased phospho-ERK1/2 levels in lysates of colonic mucosa. Immunohistochemistry revealed an expansion of the zone of phospho-ERK1/2 from the base of the crypts extending approximately 2/3 toward the lumen in Smad4 deficient, while p-ERK was restricted to the base of crypts in control mice. In addition, we observed evidence of impaired cell differentiation with decreased populations of colonic epithelial cells labeling with carbonic anhydrase II, Muc2 and chromogranin A. In conclusion, we hypothesize that Smad4 normally suppresses production of specific growth factors and cytokines that act through Erk1/2 to regulate proliferation and differentiation, and that loss of Smad4 activates these pathways leading to increased proliferation and delayed differentiation. We will test this hypothesis by inhibiting Erk1/2 activation in mice that have conditional deletion of Smad4 in intestinal epithelium using a pharmacological MEK inhibitor, comparing the responses of proliferation and differentiation in Smad4-positive compared to Smad4-depleted colonic crypts.

HHMI-70

Discovering genes of allergic disease

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Although much is known about how mammals respond to allergens, it is still unclear how allergies are acquired. Origins of allergy have both environmental and genetic causes and the incidence has been rising in recent decades in developed nations. I have taken a forward genetic approach to discover genes with nonredundant function in allergic sensitivity. A screen was developed to measure antigen-specific IgE antibody response to a model cysteine protease allergen, papain, in N-ethyl-N-nitrosourea (ENU) mutagenized mice. Males heterozygous for ENU-induced mutations (G1) are bred to wild-type females, and their daughters (G2) are backcrossed to the G1 to produce homozygous mutations in G3 mice, which are screened for IgE responses to papain. Whole exome sequencing of the G1 detects single nucleotide mutations, which are genotyped in all G3 progeny. This method allows for real-time mapping, where phenotype (papain-specific IgE antibody level) and genotype are compared statistically using dominant, additive, and recessive models of inheritance to determine whether an observed phenotype is linked to a mutation. I have screened 13,911 G3 mice in 602 pedigrees and have saturated the genome between 3.8% (null mutation 3×) and 16.8% (probably damaging or null ×3). I have identified several phenotypes that map to genes with previously unknown function in producing elevated IgE. Two of the genes have known roles in Th2 responses and were able to be detected by this screen. These mutations will be confirmed by performing either CRISPR gene knockout or replacement of the original mutation. This first forward genetic screen of allergic disease reveals that many genes may be needed to prevent the allergic response. In conclusion, these genetic discoveries may help elucidate the pathogenesis of a dysfunctional immune system in allergic disease.

HHMI-71

Inhibition of MEK and DNA damage response pathways induces synergistic killing of *Mll-Af4* B-cell acute lymphoblastic leukemia harboring activated *Ras* mutations

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Childhood B-cell acute lymphoblastic leukemias (B-ALL) that possess a translocation of the *MLL1* and *AF4* genes, are considered high-risk with poor prognosis (event-free survival

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(EFS) of 35%-50%), especially when compared to non-*MLL*-rearranged (MLL-R) childhood ALL (EFS >85%). Large-scale patient sequencing of childhood *MLL*-R leukemias showed that activating mutations of the proto-oncogene *RAS* are found in almost 50% of patients with co-occurring mutations, resulting in even poorer overall survival rate. Here, we used a recently developed model of B-ALL combining *MLL-Af4* and *RAS* mutations to assess novel therapeutic targets for patients with *MLL*-R B-ALL harboring Ras mutations. We found that Mll-Af4/*Nras* B-ALLs are responsive to single agent MEK inhibition with trametinib (GSK212), both *in vitro* and *in vivo*. Furthermore, when combined with the ATR inhibitor AZ20 *in vitro*, a significant synergy was observed at sub-IC50 concentrations. Similar studies in different tumor settings have also suggested that inhibition of the DNA damage response (DDR) represents a novel therapeutic target in cells with increased replicative stress, which is observed in our *Mll-Af4/Nras* model, due to oncogene activation and increased reliance on a functional ATR-dependent cell cycle checkpoint. Strikingly, mice with secondary B-ALL treated with trametinib and AZ20 *in vivo* had significantly prolonged survival and reduced leukemic burden in all hematopoietic tissues examined compared to vehicle or single agent alone. Inactivation of the ATR checkpoint and inhibition of MEK pathways are synergistically cytotoxic to *Mll-Af4/Nras* B-ALL cells, resulting in increased apoptosis. Overall, using our new *in vivo* murine model of *Mll-R* B-ALL, we have demonstrated a potential novel combination therapy for a group of patients with abysmal prognoses.

HHMI-CURE-72

Recapitulation of a human epilepsy syndrome in Fgf13 mutant mice

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Generalized epilepsy and febrile seizures plus (GEFS+) is a common genetic epilepsy syndrome. Mutations in four genes including *Scn1a* and *Scn1b* have been identified from large GEFS+ families. These genes account for less than 20 percent of cases and further identification of GEFS+ genes is necessary to gain insights into the genetic mechanism of epileptogenesis. Our lab has identified a family with a translocation between chromosomes X and 14 that is associated with a GEFS+ phenotype with febrile seizures and temporal lobe epilepsy. The breakpoint on the X chromosome disrupts a gene encoding FGF13, an auxiliary protein of voltage gated sodium channels. In addition, the breakpoint eliminates expression of three (V, Y, and VY) isoforms but preserves expression of two (S and U) isoforms. Our lab engineered a mouse model, in which all isoforms of FGF13 – V, Y, VY, S and U were eliminated, resulting in embryonic lethality in males and seizures typical of GEFS+ in heterozygous females. In order to understand the role of individual isoforms of FGF13, Y isoform FGF13 knockout mice were engineered resulting in mice lacking Y and VY isoforms. We have discovered that elimination of exon 1Y is sufficient to reproduce a key facet of the GEFS+ phenotype, namely age dependent hyperthermia induced seizures. This phenotype is evident in both males lacking exon 1Y and females heterozygous for exon 1Y. Currently experiments are in progress to determine if the mutant 1Y mice are epileptic.

HHMI-73

Functional evaluation of microRNA recognition elements in brain expressed transcripts

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MicroRNAs (miRNAs) are short, noncoding double-stranded RNAs that play a key role in post-transcriptional gene regulation as part of the miRNA-induced-silencing-complex (miRISC). The targeting specificity of miRNAs is driven by a requirement for close to perfect Watson-Crick base pairing between nucleotides 2-8 in the miRNA and the corresponding miRNA recognition element (MRE) in the mRNA. Although mRNA:miRNA pairing is predicted to span a variety of sequence elements, the majority of early work has focused on the functional activity of the miRNAs pairing with the 3'-untranslated region (UTR). We recently generated a transcriptome-wide map of Ago2 binding sites in mRNAs from human brain using CLIP-seq, and mapped 7,153 MREs. In addition to the expected 3'UTR binding sites, we identified a number of MREs in coding exons. Cross-referencing the human brain MREs with the Single Nucleotide Polymorphism Database identified MREs within previously reported disease-associated SNPs. After filtering, we identified 32 mRNA:miRNA interactions that we hypothesized would be disrupted or enhanced by a SNP. For functional validation, we chose 12 genes with MREs in the 3'UTR and 20 genes with MREs in the coding sequence. We found functional activity of the reported MREs in reporter assays and confirmed the functional relevance of the MRE-resident SNPs on protein expression in 3'UTRs and exons. Current experimentation is underway to test the candidate MREs and the effects of the corresponding SNPs in an endogenous setting. To this end we identified iPSC cell lines harboring the desired SNPs and plan to differentiate them into pathologically relevant cell types. Our studies are amongst the first to evaluate the functional activity of SNPs within MREs in 3'UTRs or coding exons in neural cells of human origin. These results complement earlier work identifying associations between miRNA dysregulation and neurodegenerative disorders.

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HHMI-74

Bacteriophage-mediated elimination of *Escherichia coli* and *Pseudomonas aeruginosa* for cystic fibrosis applications

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With antibiotic resistance on the rise, phage therapy is a promising alternative to antibiotics as phages offer high specificity to a range of bacterial hosts and the ability to multiply in, evolve with, and kill their hosts. In cystic fibrosis (CF), bacterial infections of the airways are common and pulmonary disease is the primary cause of morbidity and mortality. Despite aggressive antibiotic therapy, virulent bacteria can become resistant and persist, contributing to patient decline. We will investigate the *in vitro* efficacy of diverse bacteriophages against *Escherichia coli* as a model organism and transfer our approaches to CF-associated clinical isolates of *Pseudomonas aeruginosa*. Various doses and cocktails of phages will be used to understand phage and bacteria dynamics over time. Using a mass-action kinetic approach, bacterial emergence of resistance to phage therapy will be modeled to determine conditions that limit the development of phage resistance. Phages will be characterized based on sequence analysis, transmission electron microscopy (TEM), and infectivity profiles across a library of bacteria strains. Understanding the efficacy of phage therapy *in vitro* will encourage and inform future phage-based treatments for CF and other human conditions plagued by antibiotic resistance.

HHMI-75

Characterization of cytokine pathways associated with Langerhans cell facilitation of UVB-induced epidermal carcinogenesis

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Basal cell carcinomas (BCCs) and squamous cell carcinomas (SCCs) arise from damaged keratinocytes (KCs) following exposure to ultraviolet B radiation (UVB). Situated adjacent to nearly all KCs is a dendritic network of epidermal Langerhans cells (LCs). Our group previously implicated LCs in the development of UVB-induced KC cancers, with LC-deficient (huLangerin-DTA) mice being relatively resistant to tumor formation following chronic UVB irradiation. LC-intact mice were also shown to have higher epidermal expression of IL-23 and IL-22 following chronic UVB exposure as measured by quantitative PCR. Here we show that activation of toll-like receptors (TLR-2, -3, -4 and -9) on the murine LC cell line XS106 results in increased IL-23 production measured by multiplex cytokine assay. Treatment with the TLR-4 agonist LPS resulted in a 37.6 fold increase in IL-23 production ($p = .0014$), TLR-3 agonist Poly(I:C) a 4.5 fold increase ($p < .0001$), TLR-4 agonist lipoteichoic acid a 3.4 fold increase ($p = 0.0046$), and TLR-9 agonist ODN1668 a 15.4 fold increase ($p = .0061$). Notably, treatment with TLR-7 agonist imiquimod resulted in no significant change in IL-23 production. Furthermore the UVB-induced damage-associated molecular pattern (DAMP) HMGB1, which binds TLR-2, TLR-4, and TLR-9 increased production of IL-23 by freshly-isolated murine LCs 2.7 fold ($p = .0007$). IL-23 has previously been shown to induce production of the epidermal growth factor IL-22 by both type three innate lymphoid cells (ILC3s) and Th17 cells and may ultimately prove a useful target in the prevention of UVB-induced skin cancers. We also show increased production of IL-22 by ILC3 following chronic UVB exposure. Together, this data suggests a hypothetical paradigm whereby TLR activation of LCs by UVB-induced DAMPs induces IL-23 production, which subsequently signals ILC3s to produce IL-22, delivering a proliferative signal to KCs, including those harboring UVB-induced mutations

HHMI-76

Broadband power spectral density (PSD) changes observed in local field potentials (LFP) during subcallosal cingulate deep brain stimulation (SCC-DBS) for treatment resistant depression (TRD)

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Previous DBS studies suggest the importance of optimal stimulus parameters to achieve robust clinical responses. However few mechanistic hypotheses have been proposed or tested to explain these findings. To begin investigating stimulus frequency effects in SCC-DBS for TRD, we recorded SCC-LFP's to study DBS-related broadband PSD slope changes. Prior to chronic DBS therapy, 4 SCC-DBS patients (2 females, stimulated at 6mA amplitude and 1 male and 1 female stimulated at 6V) were briefly stimulated bilaterally at 130Hz, enabling pre-stimulus, stimulation and post-stimulus LFP comparisons. LFP's from the left electrode were sampled at 422Hz. Pre-stimulus (before onset) and post-stimulus epochs of 14sec were selected for analysis. Stimulation condition was omitted from this study to avoid contamination by DBS artifact. Preprocessing involved 50Hz forward-backward low-pass filtering to minimize stimulation/signal artifacts, and PSD estimation was conducted using Welch's method. PSD slopes were estimated utilizing linear regression with log(frequency) as the input variable and log(power) as the output. Differences in PSD slope characteristics between conditions were observable at several frequency bands. Pre-stimulus PSD's consistently contained major inflection points

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('knees') close to 2-3Hz, 5Hz and 12-16Hz while post-stimulus PSD's contained a single inflection close to 3Hz. Broadband PSD slope-related changes were observed in 3 SCC-DBS patients between pre-stimulus and post 130Hz-stimulation periods, suggesting the importance of broadband PSD characterization in electrophysiological studies of specific DBS parameter settings.

HHMI-SIRF-77

Hepatic thermal ablation: effect of device and heating parameters on local tissue reactions and distant tumor growth

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Thermal tumor ablation is used to treat a wide range of focal tumors, with over 100,000 treatments per year worldwide. Advantages include a lower associated morbidity and mortality compared to surgery or systemic chemotherapy, use in patients who are not surgical candidates, and cost-effectiveness. However, there is increasing awareness that local tissue reactions around the ablation zone can lead to widespread immunogenic effects. Studies have shown that non-lethal hyperthermia (40-45°C) in the periablational rim can lead to an upregulation of several angiogenic factors, tissue growth factors, and cytokines. These changes potentially have significant consequences on local tumor recurrence and distant tumor growth. The focus of our study was to investigate the biological effects between two different ablation modalities, microwave (MW) and radiofrequency (RF), and to evaluate if these changes have an impact on distant tumor growth. Using rat models, we demonstrated an increase in lymphocytic infiltration, macrophages, heat shock proteins (HSP70), and α-smooth muscle actin-positive hepatic stellate (α-SMA) cells in the periablational zone that differed by treatment modality. Additionally, there was upregulation of liver and serum levels of vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), and interleukin-6 (IL-6), based on ablation method. Lastly, we showed that both RF and MW ablation of the liver stimulated increased distant tumor growth with corresponding increased proliferative indices. These tumor growth rates varied between modalities and, in the case of MW ablation, by power level. Our study implicates that both MW and RF ablation can cause an increase in distant tumor growth through immunogenic effects. Furthermore, that these effects can be largely mitigated with higher-power, faster heating protocols. These findings can aid in the development of ablation-specific combination therapies.

HHMI-78

Mechanisms governing human memory-like natural killer cell function

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Natural killer (NK) cells are innate lymphoid cells critical for host defense against viral infection and malignant transformation. NK cells exhibit innate immunologic memory in response to specific haptens, viruses, or combined cytokine pre-activation. Human cytokine-induced memory-like NK cells, generated by overnight pre-activation with IL-12, IL-15, and IL-18, respond more robustly to numerous stimuli (including leukemia target cells) for weeks to months following the initial pre-activation. The mechanisms responsible for the enhanced effector function of human memory-like NK cells are poorly understood. We hypothesized that memory-like NK cell differentiation augments the function of anergic NK cell populations. NK cells achieve functional competence through self-MHC class I interactions (licensing) during maturation; if this does not occur, NK cells remain hypofunctional. We tested this hypothesis using normal donors with licensed and unlicensed NK cell populations. We observed that cytokine pre-activation increases IFN-γ production by both licensed and unlicensed NK cells in response to tumor targets or activating receptor (FcgRIIIa) ligation. Moreover, in response to cytokine re-stimulation, memory-like NK cells produce significantly more IFN-g than controls with no impact of licensing. We are investigating epigenetic modification of the IFN-γ gene locus as a potential molecular mechanism for these observations. Thus, the recruitment of archetypally anergic NK cell populations represents a cellular mechanism responsible for the enhanced effector function of memory-like NK cells, which may be harnessed for anti-tumor immunotherapy.

HHMI-79

Immunization against malignant melanoma in a murine model using extracorporeal photochemotherapy

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Since 1987, extracorporeal photochemotherapy (ECP) has demonstrated remarkable efficacy as a therapy for cutaneous

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T cell lymphoma (CTCL) and graft versus host disease (GVHD). Interestingly, ECP appears to provide lasting immunization against CTCL antigens while tolerizing to tissue antigens in the setting of GVHD. Given the immunomodulatory effects of this therapy, we hoped to apply its principles to the treatment of melanoma, a particularly immunogenic malignancy. To this aim, we treated C57BL/6 mice engrafted with a melanoma cell line engineered to carry three driver mutations of human melanoma: $BRAF^{V600E}$, $PTEN^{-/-}$ and $CDKN2A^{-/-}$. Treatments consisted of passing peripheral blood mononuclear cells (PBMCs) through a modified plate system adapted from human ECP and incubation of the processed PBMCs with tumor cells treated with UVA activated 8-methoxypsoralen prior to reinfusion. Mice were given six treatments over three weeks, and tumor sizes were measured twice weekly. Compared to the control group (n = 5), the treatment group (n = 5) exhibited significantly delayed tumor growth by day 27 post-inoculation ($p < 0.05$). $CD11b^+$ myeloid derivatives within the treatments appear to play an important role in controlling tumor growth as negative selection for these cell types preserved the anti-tumor response. Furthermore, *in vivo* depletion of CD3 nearly eliminates the effect, suggesting that treatments promote a T cell mediated immune response. This adaptation of ECP may facilitate antigen processing and presentation by dendritic cells *ex vivo*, thereby enhancing adaptive immunoprotection against malignant cells.

HHMI-80

Clonal hematopoiesis determination by the human androgen receptor gene (*HUMARA*) assay enriches for somatic mutations in chromatin and epigenetic regulatory genes

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Recent large retrospective studies revealed that somatic mutations in chromatin and epigenetic regulatory genes (CERGs), including *ASXL1*, *DNMT3A*, and *TET2*, occur in healthy older individuals and increase their risk for hematologic malignancy and all-cause cardiovascular deaths. CERGs play a key role in regulating DNA methylation (*DNMT3A* and *TET2*) or histone function (*ASXL1*) and in clonal proliferation of hematopoietic stem cells. We hypothesize that older women demonstrating clonal hematopoiesis, defined as a phenomenon where a hematopoietic stem cell has acquired a survival and proliferative advantage, can more accurately identify women at risk for somatic mutations in CERGs. We used the human androgen receptor gene (*HUMARA*) assay to detect non-random X-inactivation in women, a marker for clonal hematopoiesis. We tested 181 blood samples from women ≥65 years old without a history of invasive cancer or hematologic malignancy and identified samples with clonal hematopoiesis qualitatively. Only older women were used as somatic mutations increase with age and our assay depends on two X chromosomes. This was followed by confirmation via quantitative AUC analyses. Using this approach, 26% displayed definitive clonal hematopoiesis, 53% displayed definitive non-clonal hematopoiesis, and 21% displayed indeterminate patterns. Similar to previous reports, clonal hematopoiesis increased with age. Using next generation sequencing, we identified somatic mutations in CERGs in 15% of subjects with clonal hematopoiesis (3 *ASXL1* and 2 *DNMT3A* mutations with an average variant allele frequency of 15.7%). We conclude that clonal hematopoiesis in older women enriches for somatic mutations in CERGs in peripheral blood cells leading to an increased risk for hematologic malignancy and all-cause cardiovascular deaths.

HHMI-81

αA334T polymorphism enhances salt-sensitive inhibition of the epithelial sodium channel

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The epithelial sodium channel (ENaC) forms a critical pathway for sodium absorption in the renal collecting duct. Inherited mutations cause Liddle's syndrome, an autosomal dominant form of hypertension, as well as the autosomal recessive salt wasting disorder, pseudohypoaldosteronism type 1. Therefore, elucidating mechanisms that regulate ENaC may lead to a greater understanding of blood pressure control and the pathogenesis of hypertension. Previous work suggests that the ENaC extracellular domain acts as a sensor to detect and respond to a variety of signals in the extracellular environment. For example, extracellular sodium and chloride both inhibit ENaC activity, providing feedback mechanisms to modulate renal sodium absorption. We identified a common polymorphism (3.7% of European American alleles, 45% of African American alleles) in the extracellular domain of αENaC: A334T. To test the effects of this polymorphism on ENaC function, we expressed it in *Xenopus* oocytes by nuclear injection of cDNAs encoding ENaC. One day after injection, we measured ENaC sodium currents by two-electrode voltage clamp. Cells expressing the αA334T variant had smaller sodium currents than cells expressing wild type ENaC. This finding was specific to the αA334T polymorphism; mutation to other amino acids did not reduce current. In addition, cells expressing αA334T had a greater degree of sodium self-inhibition (with peak current remaining the same), as well as a greater degree of Cl^- inhibition.

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In summary, the αA334T polymorphism decreased ENaC current by increasing inhibition by sodium and Cl^-. We speculate that by enhancing salt-sensitive inhibition, this variant may protect against salt-sensitive increases in blood pressure.

HHMI-BWF-82

Hyaluronan processing and function in the progression of breast cancer

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Hyaluronan (HA) is a large, soluble, glycosaminoglycan of the extracellular matrix that has anti-inflammatory and anti-angiogenic effects under physiologic conditions. However, HA is cleaved into low molecular weight (LMW) fragments under conditions of cellular or organismal stress, acting as a molecular "switch" that promotes inflammation. In breast cancer, a decrease in HA synthesis has been correlated with decreased tumor cell proliferation and migration. However, the roles of HA fragmentation in the progression of breast cancer are unknown. We predict that HA fragmentation increases during this transition, promoting both inflammation and angiogenesis through LMW HA-CD44 interactions. To test our hypothesis, the presence/absence of HA fragmentation was determined using gel electrophoresis in breast cancer cell lines, along with healthy mammary tissue and mammary tumors derived from mice. Our data suggests as a cancerous lesion progresses, HMW HA production increases via hyaluronan synthase 2 (HAS2), but HA fragmentation does not occur until the tumor acquires a more aggressive phenotype. We are also evaluating changes in IL-6, IL-8, and VEGF gene expression as downstream effects of CD44 signaling in response to HA synthesis inhibition along with LMW and HMW HA treatments. Our data suggests that IL-8 expression decreases with the inhibition of HA synthesis, yet significant changes have not been seen between IL-6 or VEGF expression. Finally, we have verified the presence of CD44 protein in normal and cancerous cell lines via flow cytometry and found an increase in CD44 cell surface expression in aggressive tumor cells when compared to normal cells. We are currently working on knocking out CD44 using the CRISPR/Cas9 method to determine the functional relationship between the CD44-HA interactions. By targeting CD44 signaling associated with inflammation and angiogenesis, new therapeutic approaches can be developed for the treatment of breast cancer.

HHMI-83

Identifying and characterizing novel candidate genes for Andersen-Tawil syndrome

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Andersen-Tawil syndrome (ATS) is a rare genetic disorder, characterized by distinctive symptoms; episodic muscle weakness and paralysis, cardiac arrhythmias, and a variety of distinctive craniofacial and skeletal features, dental anomalies, and neurocognitive phenotype. ATS occurs sporadically or inherited as an autosomal dominant trait, with highly variable expressivity. We previously identified a gene, *KCNJ2* that is mutated in approximately 65% of all ATS families. The *KCNJ2* gene encodes the Kir2.1 protein, an inwardly rectifying potassium channel that plays a critical role in regulating the terminal phases of repolarization of the action potential in skeletal muscle and heart. All mutations occur in highly conserved and functionally important protein domains and include deletion, substitution, and de novo mutations. These mutations co-segregate with the disease in patients with a family history with variable expressivity. Also, it is found that deletion of Kir2.1 in mice causes cleft palate and digital defects, which are strikingly similar to the developmental defects of ATS patients.

However, approximately 35% of well-characterized ATS patients have no recognized *KCNJ2* mutations, suggesting that there are additional unidentified genes that cause ATS. The remaining *KCNJ2*-mutation negative patients share a similar clinical phenotype to that of *KCNJ2*-mutation positive patients. We carried out whole exome sequencing (WES) in 20 probands who do not harbor *KCNJ2* mutations, and identified several candidate genes and novel mutations with statistical significance ($p<1x10^{-5}$), which include *HAX1, CRB1, KRBA1, SEC31B*. We started with investigating the effects of the mutant *HAX1* in HEK293 cells. We generated recombinant constructs of WT and mutant *HAX1*, and *PLN*, a gene that encodes a small protein known to interact and translocate with *HAX1* protein. Our immunohistochemistry experiment demonstrated that the *HAX1* mutant protein fails to colocalize with *PLN* in the cell. We are concurrently investigating the other candidate genes, and we hypothesize that further characterization of these genes and mutations would greatly facilitate the underlying disease mechanism and pathway.

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HHMI-84

Basophils promote acute atopic dermatitis-associated itch after antigen challenge

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Atopic dermatitis (AD) is a common, relapsing inflammatory skin disorder whose most prominent and incapacitating symptom is itch, or pruritus. During a disease flare, baseline chronic itch may acutely intensify and further debilitate an individual suffering from AD. Employing a murine model of AD-like disease induced by the topical vitamin D analog MC903 (calcipotriol), we recently found that basophils accumulate in the skin and promote the development of skin thickening and histopathologic features of inflammation. However, the role of basophils in acute and/or chronic AD-associated itch remains poorly understood. In this study, we employed a basophil depletion strategy to test whether basophils contribute to AD-associated itch. Despite a reduction in AD-like disease features, itch at the primary site was unchanged. We previously showed that basophils promote sensitization to environmental antigens encountered on AD-like lesions, and elicit systemic allergic responses upon antigen challenge at a secondary site. Thus, we hypothesized that basophils may be important in mediating acute antigen-induced itch following chronic epicutaneous antigen sensitization. To test this, we sensitized mice with the model antigen ovalbumin (OVA) at the primary site of AD-like disease, and then challenged mice at a separate non-diseased site. Strikingly, OVA challenge elicited severe and sustained scratching behavior in wild-type mice that was significantly reduced in basophil-deficient littermates. Our findings reveal a previously unrecognized role of basophils in mediating acute itch flares in AD, and suggest that modulation of basophil function with targeted therapies such as omalizumab (a humanized monoclonal anti-IgE antibody) may be beneficial in reducing AD-associated acute pruritus. Towards this end, mass cytometry studies assessing basophil phenotypes in the skin of human AD patients are currently underway.

HHMI-85

Synergy between PARP and Wee1 inhibitors suggests homologous recombination repair defect in NSCLC as a mechanistic target for combination therapy

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Recent evidence suggests that impaired homologous recombination (HR) occurs in a subset of non-small cell lung cancers (NSCLCs) and may serve as a predictive biomarker for sensitivity to DNA damaging agents. Poly-ADP ribose polymerase (PARP) and Wee1 inhibition represent two mechanistically distinct approaches to augment the effects of DNA damage. Specifically, the PARP inhibitor olaparib is synthetic lethal in HR deficient tumors. AZD1775 is a Wee1 inhibitor that abrogates the G2/M checkpoint and has been reported to exhibit single-agent activity in patients harboring BRCA1/2 mutations. Therefore, we hypothesize that olaparib and AZD1775 would have synergistic effects in a subset of NSCLCs and that HR deficiency could be predictive of tumor response. Utilizing Rad51 focus formation as a marker of HR deficiency, we prospectively selected representative NSCLC cell lines that either did (e.g. Calu6) or did not (e.g. A549) harbor putative defects in HR repair. We treated Calu6 and A549 and other NSCLC cells with varying doses and schedules of AZD1775 and olaparib, and determined cytotoxicity and effect on downstream protein markers. In response to combination treatment, Calu6 cells demonstrated markedly more pronounced synergistic sensitivity (median CI = 0.19) compared to A549 cells (median CI = 0.90). On biochemical analysis, we observed inhibition of p-Cdk1, upregulation of p-Chk1, and upregulation of p-KAP1, suggesting abrogation of the G2/M checkpoint and activation of ATM/ATR repair pathways, all consistent with the mechanistic underpinnings of our hypothesis. Taken together, these results provide early pre-clinical evidence for the rational combination of Wee1 and PARP inhibition in the treatment of advanced NSCLC, and suggest HR deficiency as a predictive marker applicable to NSCLC. Continued mechanistic investigation and further confirmatory studies are warranted to inform the selection of patients who may maximally benefit from such combination treatment.

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HHMI-86

Mechanism of enhanced angiotensin II-mediated renal vasoconstriction in spontaneously hypertensive rats

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Development of essential hypertension is intimately related to impaired renal hemodynamics. Spontaneously hypertensive rats (SHR), compared to normotensive Wistar-Kyoto rats (WKY), exhibit enhanced renal vascular resistance (RVR) in response to angiotensin II (Ang II), a Gq-coupled receptor agonist. This response can be further augmented in SHR with Gi-coupled receptor agonists, and can be normalized by inhibiting the Gi pathway with pertussis toxin (PT). These data show that enhanced Ang II-mediated renal vasoconstriction in SHR requires Gi signaling; however, mechanisms governing this phenomenon are poorly understood. Past studies suggest that increased afferent arteriolar vasoconstriction by pre-glomerular vascular smooth muscle cells (PGVSMCs) may be responsible. We found that SHR PGVSMCs cultured and embedded in a collagen gel matrix demonstrate enhanced contraction in response to Ang II compared to WKY PGVSMCs. The addition of neuropeptide Y (NPY), a physiologic Gi agonist, further augments this response in SHR PGVSMCs, but not in WKY PGVSMCs. Moreover, inhibition of Gi signaling with PT decreases SHR PGVSMC contraction similar to levels observed in WKY PGVSMCs. These *in vitro* findings correlate well with past *in vivo* observations. To delineate a molecular mechanism, we looked toward Gq and Gi signal transduction. The Gq and Gi pathways, via the Gαq and Gβγ G protein subunits, respectively, converge on phospholipase C-β3 (PLCβ3) to initiate contractile signaling. We found that membrane isolates of SHR PGVSMCs possess significantly more Gαq, Gβγ, and PLCβ3 compared to WKY PGVSMCs. Additionally, selective inhibition of PLCβ with the small molecule U73122 completely prevents contractile responses, supporting PLCβ3 as a crucial point of convergence. Our results demonstrate that greater PGVSMC contractile responses are likely responsible for enhancing Ang II-mediated renal vasoconstriction in SHR, due to greater membrane localization of Gq and Gi signal transducers.

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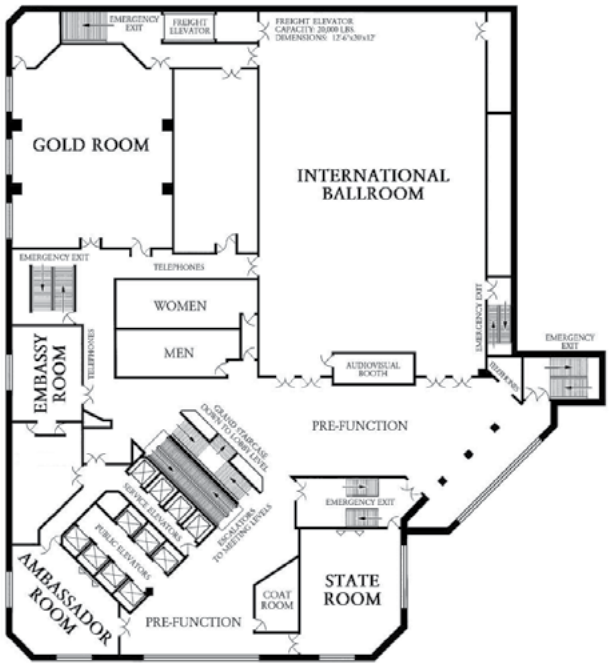

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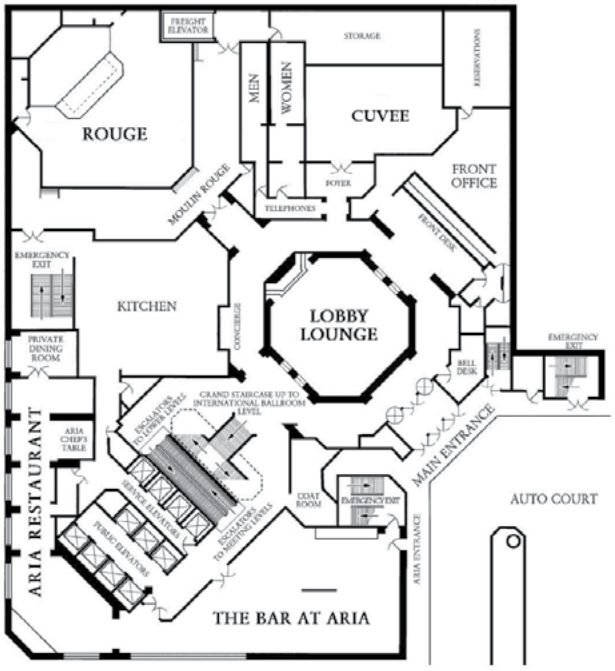
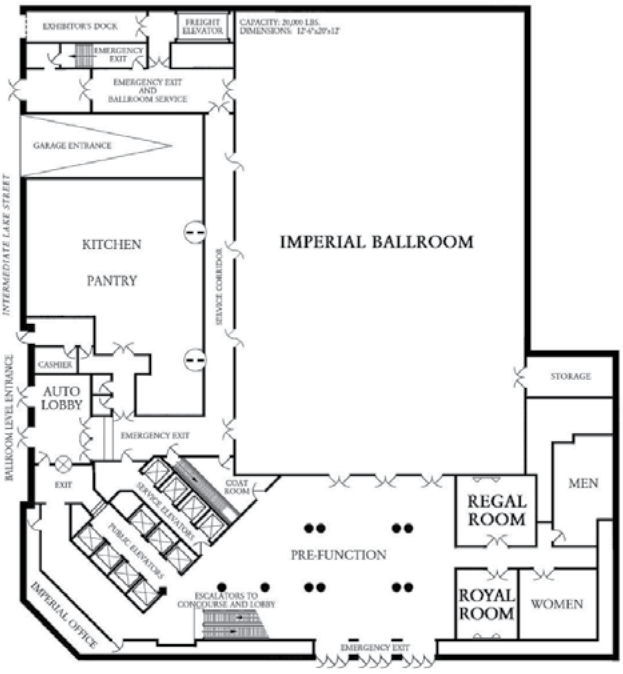


Imperial Ballroom Level (B2 level)



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