AAP/ASCI/APSA Joint Meeting 2017

Meeting Program & Abstracts

AAP/ASCI/APSA Joint Meeting 2017

THE PREMIER ANNUAL MEETING FOR PHYSICIAN-SCIENTISTS

APSA
American Physician Scientists Association

April 21 – 23, 2017

Fairmont Chicago Millennium Park

Chicago, Illinois

HARVARD MEDICAL SCHOOL

This activity is jointly provided by Harvard Medical School.

www.jointmeeting.org

Special Events At The 2017 AAP/ASCI/APSA Joint Meeting

Friday, April 21

ASCI President's Reception

6:15 p.m. – 7:15 p.m.
Gold Room

ASCI Dinner & New Member Induction Ceremony

(Ticketed event)

7:30 p.m. – 9:45 p.m.
Rouge, Lobby Level

How to Earn a Nobel Prize
Speaker: **Michael S. Brown, MD**
UT Southwestern Medical Center

APSA Welcome Reception

(Ticketed event, ID required)

9:00 p.m. – Midnight
Mid-America Club, Aon Center *(off-site)*

American Physician Scientists Association

The Premier Annual Meeting For Physician-Scientists

Saturday, April 22

ASCI Food & Science Evening

(ID required)

6:30 p.m. – 9:00 p.m.
Mid-America Club, Aon Center *(off-site)*

Featuring Poster Presentations by the ASCI's 2017 Young Physician-Scientist Award Recipients and Howard Hughes Medical Institute Medical Research Fellows.

AAP Member Banquet

(Ticketed event)

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

Can Science Avert the Decline and Fall of the American Empire?
Speaker: **Roberta B. Ness, MD, MPH**
University of Texas School of Public Health

APSA Dinner & Founder's Award Presentation

(Ticketed event)

7:30 p.m. – 9:00 p.m.
Rouge, Lobby Level

Founder's Award Recipient: **Joseph Bast, PhD**
Founding Director, ASPA Board of Directors

Charting Your Path as a Physician Scientist
Dinner Speaker: **Kirsten Bibbins-Domingo, MD, PhD, MAS**
University of California, San Francisco

Dessert Reception

(Open to all attendees)

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

Sunday, April 23

APSA Residency Luncheon

12:00 p.m. – 2:00 p.m.
Rouge, Lobby Level

Program Contents

General Program Information

Registration Desk Hours

Friday, April 21	7:00 a.m. – 6:30 p.m.
Saturday, April 22	7:00 a.m. – 5:00 p.m.
Sunday, April 23	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 21

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 22
TWO Poster Presentation Sessions

8:00 a.m. – 9:00 a.m.	Poster Session with Continental Breakfast **ODD numbered posters presented**
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 presenters should plan to be available on Saturday for their appointed poster presentation session and the resulting awards program later in the afternoon.

Best 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 22, from 2:45 – 3:00 p.m.

› Continuing Medical Education Information

Continuing Medical Education (CME) Information

The AAP/ASCI/APSA Joint Meeting annually brings together physician-scientists of all backgrounds to highlight some of the best examples of translational biomedical research, to celebrate new members elected to the AAP and ASCI in recognition of their research and leadership contributions, and to recognize the careers of physician-scientists who have had major impact on their fields.

Meeting Learning Objectives

Upon completion of this activity, participants will be able to:

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

Target Audience

This activity is targeted towards physician-scientists, trainees and students across a broad range of specialties including basic research, cardiology/cardiovascular research, cell and molecular biology, endocrine and metabolism, hematology, immunology, infectious diseases, nephrology, pulmonology, and others.

HMS Disclosure Policy

HMS adheres to all Accreditation Council for Continuing Medical Education (ACCME) Accreditation Criteria and Policies. It is HMS's policy that those who have influenced the content of a CME activity (e.g. planners, faculty, authors, reviewers and others) disclose all relevant financial relationships with commercial entities so that HMS may identify and resolve any conflicts of interest prior to the activity. These disclosures will be provided in the activity materials for the learners along with disclosure of any commercial support received for the activity prior to the beginning of the activity.

HMS strives to ensure that the content of all its accredited activities is independent from any commercial influence, presents a balance of therapeutic options, promotes improvements in healthcare and is in alignment with the ACCME Content Validation Policy. Additionally, faculty members have been instructed to disclose any limitations of data and unlabeled or investigational uses of products, pharmaceuticals or medical devices during their presentations.

Accreditation Statement

This activity has been planned and implemented in accordance with the accreditation requirements and policies of the Accreditation Council for Continuing Medical Education (ACCME) through the joint providership of Harvard Medical School and the Association of American Physicians, the American Society for Clinical Investigation, and the American Physician Scientists Association. The Harvard Medical School is accredited by the ACCME to provide continuing medical education for physicians.

AMA Credit Designation Statement

The Harvard Medical School designates this live activity for a maximum of 7.50 *AMA PRA Category 1 Credits*™. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

Claiming CME Credit and Obtaining Certificates

Participants will be sent a link to the online evaluation before the start of the meeting and will be required to complete the online evaluation to receive CME credit. Once the evaluation is completed, participants may print, save, or email their CME certificates or certificates of attendance (for non-physicians). Questions related to this activity or on completing this online evaluation may be directed to ceinhospital@hms.harvard.edu.

Disclaimer

CME activities sponsored by Harvard Medical School are offered solely for educational purposes and do not constitute any form of certification of competency. Practitioners should always consult additional sources of information and exercise their best professional judgment before making clinical decisions of any kind. The content of each presentation does not necessarily reflect the views of Harvard Medical School.

Joint Program Planning Committee & APSA Events Committee

Joint Program Planning Committee

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Cleveland Clinic

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Lori Ennis

Executive Director ASCI, Ex Officio Committee Member
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Events Committee Member
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University of Alabama at Birmingham

› Scientific Program Schedule

Friday, April 21, 2017

Time	Event	Location
8:30 a.m. – 11:00 a.m.	**APSA Business Meeting** *(Open to all APSA members)*	Rouge
11:00 a.m. – 1:00 p.m.	**APSA Session I**	International Ballroom
11:00 a.m. – 11:45 a.m.	 **Translating Neuroscience: Obstacles and Opportunities** APSA Speaker: **Kafui Dzirasa, MD, PhD** *Duke University Medical Center*	International Ballroom
12:00 p.m. – 12:45 p.m.	 **The Role of the Physician Scientist in Interventional Radiology** APSA Speaker: **Sanjay Misra, MD** *Mayo Clinic (Sponsored by RSNA)*	International Ballroom
1:00 p.m. – 3:00 p.m.	**Poster Setup**	Imperial Ballroom
1:00 p.m. – 6:00 p.m.	**Plenary Session I: Healthy Brain, Healthy Living** Moderators: **Alex Adami, Vivian G. Cheung, and Serpil Erzurum**	International Ballroom
1:00 p.m. – 1:30 p.m.	 **Population-Based Strategies for Promotion of Cognitive Resilience** Speaker: **Kristine Yaffe, MD** *University of California, San Francisco*	International Ballroom
1:30 p.m. – 2:00 p.m.	 **The White House BRAIN Initiative: Revolutionizing Brain Health through Innovative Technologies** Speaker: **Sarah H. Lisanby, MD** *NIH, National Institute of Mental Health*	International Ballroom
2:00 p.m. – 3:00 p.m.	**2017 ASCI and AAP Invited New Member Presentations**	
2:00 p.m. – 2:15 p.m.	 **NETting the Web in Systemic Autoimmunity** AAP New Member: **Mariana Kaplan, MD** *NIH, National Institute of Arthritis and Musculoskeletal and Skin Diseases*	International Ballroom
2:15 p.m. – 2:30 p.m.	 **Genetic Approaches to Understanding the Pathogenesis of 15q13.3 Microdeletion Syndrome** ASCI New Member: **Christian P. Schaaf, MD, PhD** *Baylor College of Medicine* *Recipient, 2016 Seldin~Smith Award for Pioneering Research*	International Ballroom
2:30 p.m. – 2:45 p.m.	 **FGF23: From Mendelian Obscurity to Mainstream Mechanism of Cardio-Renal Disease** AAP New Member: **Myles Wolf, MD, MMSc** *Duke University*	International Ballroom
2:45 p.m. – 3:00 p.m.	 **Immune Responses in Gastrointestinal Tissues** ASCI New Member: **Aida Habtezion, MD, MSc** *Stanford University*	International Ballroom
3:00 p.m. – 3:30 p.m.	**Break**	

Friday, April 21, 2017 *(continued)*

3:30 p.m. – 6:00 p.m.	Moderators: **Benjamin Ebert, Linda P. Fried, and Jillian Liu**	
3:30 p.m. – 4:00 p.m.	 **ASCI/Stanley J. Korsmeyer Award Lecture** Recipient: **James E. Crowe, Jr., MD** *Vanderbilt University*	International Ballroom
4:00 p.m. – 4:30 p.m.	 **The Biology of Memory and Age Related Memory Loss** APSA Keynote: **Eric R. Kandel, MD** *Columbia University*	International Ballroom
4:30 p.m. – 5:00 p.m.	 ASCI Presidential Address: **Vitalizing Physician-Scientists: It's Time to Overcome Our Imagination Fatigue** **Vivian G. Cheung, MD** *Howard Hughes Medical Institute, University of Michigan*	International Ballroom
5:00 p.m. – 5:30 p.m.	**ASCI / Harrington Prize for Innovation in Medicine Lecture**  **Daniel J. Drucker, MD** *Lunenfeld-Tanenbaum Research Institute of the Mount Sinai Hospital*  **Joel F. Habener, MD** *Massachusetts General Hospital, Harvard Medical School*  **Jens J. Holst, MD, DMSc** *University of Copenhagen*	International Ballroom
5:30 p.m. – 6:00 p.m.	 **Drugs, Neurotransmitters and the Brain** Lasker / APSA Lecture: **Solomon Snyder, MD, DSc, DPhil** *Johns Hopkins University*	International Ballroom
6:00 p.m. – 7:00 p.m.	**APSA Local Chapter and Institutional Representative Meeting**	State Room
6:15 p.m. – 7:15 p.m.	**ASCI President's Reception**	Gold Room
6:15 p.m. – 9:30 p.m.	**Poster Viewing Only**	Imperial Ballroom
7:00 p.m. – 9:00 p.m.	**AAP Offsite President's Dinner** *(By invitation only)*	
7:30 p.m. – 9:45 p.m.	 **ASCI Dinner & New Member Induction Ceremony** **How to Earn a Nobel Prize** Speaker: **Michael S. Brown, MD** *UT Southwestern Medical Center* *(Ticketed event)*	Rouge
9:00 p.m. – Midnight	**APSA Welcome Reception** *(Ticketed event, ID required)*	Mid-America Club

Scientific Program Schedule

Saturday, April 22, 2017

Time	Event	Location
7:00 a.m. – 8:00 a.m.	**AAP Council Meeting**	State Room
7:00 a.m. – 8:00 a.m.	**Mentoring Breakfast** *(Ticketed event)*	Rouge
8:00 a.m. – 9:00 a.m.	**Poster Session and Continental Breakfast.** *ODD number posters will be presented/judged.*	Imperial Ballroom
8:00 a.m. – 9:00 a.m.	**APSA Board of Directors Meeting**	Embassy Room
9:00 a.m. – 11:45 a.m.	**Plenary Session II: Visualizing Medicine** Moderators: **Vivian G. Cheung, Mariam Bonyadi Camacho, and Christine E. Seidman**	International Ballroom
9:00 a.m. – 9:30 a.m.	 **Charting Our Future Together: Turning Discovery into Health** Speaker: **Gary H. Gibbons, MD** *NIH, National Heart, Lung, and Blood Institute*	International Ballroom
9:30 a.m. – 10:00 a.m.	 **Visualizing GPCR – Transducer Complexes Elucidates Evolving Signaling Paradigms** Speaker: **Robert J. Lefkowitz, MD** *Duke University Medical Center*	International Ballroom
10:00 a.m. – 10:15 a.m.	 APSA Trainee Oral Abstract Presentation: **Loss of Function Mutations in GALNT14 Predispose to IgA Nephropathy** Speaker: **Sindhuri Prakash** *Rutgers University – New Jersey Medical School, ASN Travel Awardee*	International Ballroom
10:15 a.m. – 10:45 a.m.	 **Biology of Bedtime: Moving Towards an Understanding of Sleep** Speaker: **Amita Sehgal, PhD** *Perelman School of Medicine at the University of Pennsylvania*	International Ballroom
10:45 a.m. – 11:15 a.m.	 **Pathways Regulating Stem Cell Self Renewal and Migration** APSA Keynote Speaker: **Leonard I. Zon, MD** *Boston Children's Hospital, Harvard Medical School*	International Ballroom
11:15 a.m. – 11:45 a.m.	 **Presentation of the 2017 Donald Seldin~Holly Smith Award for Pioneering Research** Recipient: **Omar Abdel-Wahab, MD** *Memorial Sloan Kettering Cancer Center* **Starting Out as a Physician-Scientist: The Crucial First Step on the Ladder to Success** Introductory Remarks: **Joseph L. Goldstein, MD** *UT Southwestern Medical Center*	International Ballroom
11:45 a.m. – 1:30 p.m.	**Poster Session 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

Saturday, April 22, 2017 *(continued)*

1:30 p.m. – 4:30 p.m.	**Plenary Session III: Areas Requiring Urgent Responses** Moderators: **John Carethers, Hanna Erickson, and Kieren Marr**	International Ballroom
1:30 p.m. – 2:00 p.m.	 **Vaccine Development for Zika Virus** Speaker: **Dan H. Barouch, MD, PhD** *Beth Israel Deaconess Medical Center, Harvard Medical School*	International Ballroom
2:00 p.m. – 2:15 p.m.	 **APSA Trainee Oral Abstract Presentation: Modification of LPS by EptB Inhibits Intelectin Binding and Increases Systemic Inflammation During Salmonella Infection** Speaker: **Lillian Zhang** *University of California, Davis, School of Medicine, AAP/ASCI Travel Awardee*	International Ballroom
2:15 p.m. – 2:45 p.m.	 **Microbial Diagnostics, Surveillance and Discovery in Acute and Chronic Diseases** Speaker: **W. Ian Lipkin, MD** *Columbia University*	International Ballroom
2:45 p.m. – 3:00 p.m.	**Best Poster Awards**	International Ballroom
3:00 p.m. – 3:30 p.m.	**Break**	
3:30 p.m. – 5:45 p.m.	Moderators: **Hossein Ardehali, Brandon Fox, and Linda P. Fried**	International Ballroom
3:30 p.m. – 4:00 p.m.	 **Preparing for the Next Pandemic** Speaker: **Tadataka Yamada, MD** *Frazier Healthcare Partners*	International Ballroom
4:00 p.m. – 4:30 p.m.	 **Global Health Security: Urgent Protection Imperatives** Speaker: **Julie L. Gerberding, MD, MPH** *Merck & Co., Inc. (Sponsored by IDSA)*	International Ballroom
4:30 p.m. – 5:00 p.m.	 AAP Presidential Address: **Generational Trajectories and Public Good of Physician-led Science for Health** **Linda P. Fried, MD, MPH** *Columbia University, Mailman School of Public Health*	International Ballroom
5:00 p.m. – 5:45 p.m.	**Kober Medal Presentation**  Recipient: **Laurie H. Glimcher, MD** *Dana-Farber Cancer Institute*  Presenter: **Carl Nathan, MD** *Weill Medical College of Cornell University*	International Ballroom

Saturday, April 22, 2017 *(continued)*

6:00 p.m. – 7:00 p.m.	**APSA Panel: Team-Based Science** Moderator: **Lillian Zhang** Panelists:  **Julie L. Gerberding, MD, MPH** *Merck & Co., Inc.*  **David Meltzer, MD, PhD** *University of Chicago*  **Eric Svensson, MD, PhD** *Novartis Institutes for Biomedical Research*	Crystal Room
6:30 p.m. – 9:00 p.m.	**ASCI Food & Science Evening** *Featuring Poster Presentations by the ASCI's 2017 Young Physician-Scientist Award Recipients and Howard Hughes Medical Institute Medical Research Fellows* *(ID required)*	Mid-America Club
7:00 p.m. – 10:00 p.m.	 **AAP Member Banquet: Can Science Avert the Decline and Fall of the American Empire?** Speaker: **Roberta B. Ness, MD, MPH** *University of Texas School of Public Health* *(Ticketed event)*	Imperial Ballroom
7:30 p.m. – 9:00 p.m.	**APSA Dinner & Founder's Award Presentation** *(Ticketed event)*  Founder's Award Recipient: **Joseph Bast, PhD** *Founding Director, APSA Board of Directors*  **Charting Your Path as a Physician Scientist** Dinner Speaker: **Kirsten Bibbins-Domingo, MD, PhD, MAS** *University of California, San Francisco*	Rouge
10:00 p.m. – Midnight	**Dessert Reception** *(Open to all attendees)*	Imperial Lobby

Scientific Program Schedule

Sunday, April 23, 2017

Time	Event	Location
8:00 a.m. – 12:00 p.m.	**APSA Session II**	*Various*
8:00 a.m. – 9:30 a.m.	**Mentoring Breakfast – Medical Specialties** *(Ticketed event)*	International Ballroom
8:30 a.m. – 10:00 a.m.	**Society Leadership Wrap Up Meeting** *(By invitation only)*	Embassy Room
9:30 a.m. – 10:00 am	 **Genetic Influences on Rheumatoid Arthritis** APSA Keynote Speaker: **S. Louis Bridges, MD, PhD** *University of Alabama at Birmingham* *(Sponsored by ACR)*	Gold Room
10:00 a.m. – 11:00 a.m.	**APSA Panel: Work/Life Balance** Moderator: **Michelle Caunca** Panelists:  **Alessia Fornoni, MD, PhD** *Peggy and Harold Katz Family Drug Discovery Center*  **David Ostrow, MD, PhD** *David Ostrow & Associates, LLC*  **Karen Sibert, MD** *University of California, Los Angeles*	Gold Room
11:00 a.m. – 12:00 p.m.	**APSA Panel: Transitioning** Moderator: **Jason J. Siu** Panelists:  **Steve Freedman, MD, PhD** *Beth Israel Deaconess Medical Center,* *Harvard Medical School*  **Neelroop Parikshak, MD, PhD** *University of California, Los Angeles*  **Hui-Zi Chen, MD, PhD** *Ohio State University*  **Peter Preusch, PhD** *National Institute of General Medical Sciences*	Gold Room

Sunday, April 23, 2017 *(continued)*

11:00 a.m. – 12:00 p.m.	**APSA Panel: Dos and Don'ts of MSTP Admissions** Moderator: **Lillian Zhang** Panelists:  **Skip Brass, MD, PhD** *University of Pennsylvania*  **Chris Williams, MD, PhD** *Vanderbilt University*  **Kerry O'Banion, MD, PhD** *University of Rochester*	Embassy Room
12:00 p.m. – 2:00 p.m.	**APSA Residency Luncheon**	Rouge
	Beth Israel Deaconess Medical Center Physician - Scientist Track – *Dr. Steven Freedman*	
	University of Pennsylvania Physician Scientist Residency Program – *Dr. Peter Klein*	
	Massachusetts General Internal Medicine Program – *Dr. Jatin Vyas*	
	University of Iowa Carver College of Medicine Physician Scientist Training Pathway *Dr. Joel Kline & Dr. David Stoltz*	
	Vanderbilt University School of Medicine Physician Scientist Training Program *Dr. Christopher Williams*	
	Brigham and Women's Hospital Internal Medicine Residency Program – *Dr. Joel Katz*	
	NIH Clinical Center, Office of Clinical Research Training and Medical Education *Dr. Frederick Ognibene*	
	Children's Hospital Los Angeles George Donnell Society for Pediatric Scientists *Dr. Brent Polk*	
	The Ohio State University Physician Scientist Training Program – *Dr. Robert Baiocchi*	
	UCLA Specialty Training and Advanced Research (STAR) Program – *Dr. Tamer Sallam*	
	University of Minnesota Physician Scientist Training Program – *Dr. Clifford Steer*	
	University of Alabama at Birmingham Medicine Scholar's Program – *Dr. Sonya Heath*	
	Baylor University Pediatrician - Scientist Training and Development Program – *Dr. Audrea Burns*	
	Cincinnati Children's Pediatric Residency Research Program – *Dr. Sonya Tang Girdwood*	
2:00 p.m. – 4:30 p.m.	**APSA Research Pathway Residency Program Directors Meeting**	State Room

Speaker Biographies

Dan Barouch, MD, PhD

Dr. Barouch received his PhD in immunology from Oxford University and MD from Harvard Medical School. Dr. Barouch is presently director of the Center for Virology and Vaccine Research at Beth Israel Deaconess Medical Center and Professor of Medicine at Harvard Medical School. He is a key part of the Bill & Melinda Gates Foundation Collaboration for AIDS Vaccine Discovery, the National Institutes of Health Center for HIV/AIDS Vaccine Immunology and Immunogen Discovery, and the Ragon Institute of MGH, MIT, and Harvard.

Dr. Barouch's laboratory focuses on studying the immunology and virology of HIV-1 infection and developing novel vaccine strategies. His laboratory has explored a series of novel vaccine technologies, including adjuvanted DNA vaccines, poxvirus vectors, and alternative serotype adenovirus vectors in both preclinical and clinical studies. In particular, he has advanced a series of novel adenovirus vector-based HIV-1 vaccine candidates from concept and design to preclinical testing to phase 1 clinical trials that are currently underway in both the U.S. and sub-Saharan Africa.

Dr. Barouch is board certified in internal medicine and infectious diseases, and he is committed to mentoring students, clinical fellows, research fellows, and junior faculty and to providing clinical care to patients with infectious diseases.

Kirsten Bibbins-Domingo, MD, PhD, MAS

Dr. Bibbins-Domingo is the Lee Goldman, MD Endowed Chair in Medicine and Professor of Medicine and of Epidemiology and Biostatistics at the University of California, San Francisco (UCSF). She is the Director of the UCSF Center for Vulnerable Populations at Zuckerberg San Francisco General Hospital, a research center focused on discovery, innovation, policy and advocacy, and community engagement for populations at risk for poor health and inadequate healthcare. Dr. Bibbins-Domingo is a national expert in cardiovascular disease epidemiology; in racial, ethnic, and income disparities in health; and clinical and public health interventions aimed at chronic disease prevention. She has led many NIH grants and has been continuously NIH funded since 2004.

Dr. Bibbins-Domingo received her MD, PhD in Biochemistry, and Masters in Epidemiology and Biostatistics from UCSF, where she also completed an internship, residency, and fellowship in internal medicine. She is a practicing general internist at Zuckerberg San Francisco General Hospital.

Dr. Bibbins-Domingo has been the recipient of many honors and awards, and was appointed Chair of the U.S. Preventive Services Task Force in March 2016 and has been a member since 2010.

Lawrence (Skip) Brass, MD, PhD

Dr. Brass is a graduate of Harvard College and Case Western Reserve University, where he received his MD and a PhD in Biochemistry. He is currently Professor of Medicine and Professor of Systems Pharmacology and Translational Therapeutics. He has led the NHLBI-funded Hematology Research Training Program since 1994. Dr. Brass became Associate Dean for Combined Degree and Physician Scholars Programs and Director of Penn's MSTP in 1998. He has been active at the national level in the development of training programs for physician-scientists. He is also a practicing hematologist whose research interests are in the fields of hemostasis and vascular biology. He has been continuously funded by the NIH NHLBI since the mid-1980s, has been elected to membership in the American Society for Clinical Investigation and the Association of American Physicians, was an Established Investigator of the American Heart Association and is a recipient of the Distinguished Career Award from the International Society of Hemostasis and Thrombosis, the Christian R. and Mary F. Lindback Award for Distinguished Teaching from the University of Pennsylvania, and the inaugural Bert Shapiro Award for Leadership, Dedication and Service to the Physician-Scientist Community from the National Association of MD/PhD Programs.

S. Louis Bridges, Jr., MD, PhD

Dr. Bridges is Anna Lois Waters Endowed Professor of Medicine at the University of Alabama at Birmingham and has served as Director of the UAB Division of Clinical Immunology and Rheumatology since 2009. He is also the Director of the UAB Comprehensive Arthritis, Musculoskeletal, Bone, and Autoimmunity Center. His research has been continuously funded by NIH throughout his career and is focused on the identification of genetic influences on rheumatoid arthritis susceptibility and severity, particularly in African-Americans, autoantibodies in RA, biomarkers of treatment response in RA. He serves as Principal Investigator of the NIH-funded UAB Multidisciplinary Clinical Research Center, Co-Director of the NIH-funded UAB Center of Research Translation in Gout and Hyperuricemia, and Associate Director of the NIH-funded UAB Rheumatic Disease Cores Center. He has been named to the Best Doctors in America list yearly since 2003, is Director of UAB's NIH-funded Training Program in Rheumatic and Musculoskeletal Diseases Research, and is a Co-Editor of *Arthritis & Rheumatology.* Dr. Bridges serves as Chair of the American College of Rheumatology's Committee on Research and is a member of the Board of Directors and Scientific Advisory Council of the Rheumatology Research Foundation.

Speaker Biographies

Michael S. Brown, MD

Dr. Brown received an MD degree 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. He is currently 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. Dr. Brown and his colleague, Dr. Joseph L. Goldstein, 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.

Hui-Zi Chen, MD, PhD

Dr. Chen was born in Shanghai and lived in China until the age of ten. She moved to Lund, Sweden, where her father completed his graduate studies in the Department of Medicine at the University of Lund. In 2005, Huizi received her B.A. in Molecular and Cellular Biology from the College of Arts and Sciences at Cornell University in Ithaca, New York. After college, she came back to Columbus to attend OSU to begin her training as a physician scientist. In May 2013, Huizi graduated from MSTP and matched with the Internal Medicine PSTP Residency program at OSU. She completed her residency in 2015 and is board certified in Internal Medicine. Huizi is currently a second year fellow in the Division of Medical Oncology and performs her research under the mentorship of Dr. Sameek Roychowdhury. Her work focuses on translational genomics, the utilization of research autopsy to dissect tumor heterogeneity, and generating patient-derived xenograft models to identify mechanisms of acquired drug resistance as well as to facilitate development of new therapies for advanced cancer.

Vivian G. Cheung, MD

Dr. Cheung is an HHMI Investigator, and a Professor of Pediatric Neurology, and Human Genetics at the University of Michigan. She received her undergraduate degree from UCLA and her medical degree from Tufts University. She was a pediatric resident at UCLA, and a neurology fellow at The Children's Hospital of Philadelphia. Dr. Cheung's laboratory studies genetic variation in gene regulation. She and the late Dr. Richard Spielman showed that expression levels of human genes can be studied as quantitative traits. This enabled mapping of gene regulators without a priori knowledge of the underlying mechanisms, and facilitated the identification of regulatory variants that affect disease susceptibility. More recently, it has led her group to a surprising finding of differences between RNA and its corresponding DNA sequences beyond the known RNA editing mechanisms. They found all 12 types of RNA-DNA sequence differences (RDDs), and showed that RDD is conserved from yeast to human and linked to R-loop. Similar to alternate splicing, RDD allows a DNA sequence to form two or more transcripts. Dr. Cheung is a recipient of the Curt Stern Award from the American Society of Human Genetics. She is a member of the National Academy of Medicine. This year, Dr. Cheung is the President of the ASCI.

James E. Crowe, Jr., MD

Dr. Crowe is Director of the Vanderbilt Vaccine Center and the Ann Scott Carell Professor of Pediatrics, Pathology, Microbiology and Immunology at Vanderbilt University Medical Center. His laboratory studies the human immune response to infection or vaccination. His team has studied immunity to a wide variety of major human pathogens, including avian influenza, chikungunya virus, Ebola and Marburg viruses, dengue and Zika viruses and others. His team has developed new methods for rapid and efficient isolation of broad and potent neutralizing human monoclonal antibodies from human survivors of viral diseases. Antibodies to four viral targets from this program are now in clinical development. His group also is working to develop novel methods in computational immunology for rational structure-based design of vaccines and antibodies. He is the Director of the Human Immunome Project, an ambitious effort to identify the sequence of all transcripts for adaptive immune receptors on the planet. He is an elected member of the National Academy of Medicine and has been the recipient of a number of major investigator awards for research.

Daniel J. Drucker, MD

Dr. Drucker received his MD from the University of Toronto in 1980, and is currently Professor of Medicine at the University of Toronto. He holds a Canada Research Chair in Regulatory Peptides and the Banting and Best Diabetes Centre-Novo Nordisk Chair in Incretin Biology. His laboratory is based in the Lunenfeld Tanenbaum Research Institute at Mt. Sinai Hospital and studies the molecular biology and physiology of the glucagon-like peptides. Dr. Drucker's scientific studies identified multiple novel mechanisms of gut hormone action, resulting in 33 issued US patents, and enabling development of new drug classes for diabetes, obesity and intestinal failure. His discoveries have been recognized by numerous learned societies including the Banting Award from the ADA, the Claude Bernard Award from the EASD, the Manpei Suzuki International Prize for Diabetes Research, the Rolf Luft Award from the Karolinska Institute, and election to Fellowship in the Royal Society, London.

Kafui Dzirasa, MD PhD

Dr. Dzirasa is the first African American to complete a PhD in Neurobiology at Duke University. His research interests focus on understanding how changes in the brain produce neurological and mental illness. He was subsequently appointed as an assistant professor and house staff in the Department of Psychiatry and Behavioral Science at Duke University School of Medicine.

Kafui has served on the Board of Directors of the Student National Medical Association: a national organization dedicated to the eradication of health care disparities. Through his service as Chapter President, Kafui has participated in numerous programs geared towards exposing youth to science and technology, providing health education for underserved communities, and organizing clinics to screen for chronic diseases. Kafui received the Charles Johnson Leadership Award in 2007, and he was recognized as one of Ebony magazine's 30 Young Leaders of the Future in February 2008. He has also been awarded the International Mental Health Research Organization Rising Star Award, the Sydney Baer Prize for Schizophrenia Research, and his laboratory was featured on CBS *60 Minutes* in 2011. In 2016, he was awarded the inaugural Duke Medical Alumni Emerging Leader Award and the Presidential Early Career Award for Scientists and Engineers.

Speaker Biographies

Alessia Fornoni, MD, PhD

Dr. Fornoni obtained her MD and her PhD degree in Medical Pharmacology at the Universita' degli Studi di Pavia (Italy). She later joined the laboratory of Renal Cell Biology (Vascular Biology Institute) at the University of Miami, where she worked on animal models of diabetic nephropathy and on the role of mesangial stem cells progenitors under the direct supervision of Drs. Liliane and Gary Striker. After her post-doctoral fellowship, Dr. Fornoni completed the Internal Medicine and Nephrology training at the University of Miami (Basic Scientist Investigator pathway). She is Board Certified in both Internal Medicine and Nephrology. She currently devotes 75% of her time to research and 25% to patient care. Dr. Fornoni's research interest is focused on proteinuria and kidney diseases, with particular interest in diabetic nephropathy and focal and segmental glomrulosclerosis. Dr. Fornoni is currently interested in the intracellular signaling pathways modulated by intracellular lipids. Dr. Fornoni's research goal is to translate her basic science findings into clinical research through the identification of new therapeutic targets for chronic kidney disease.

Steven D. Freedman MD, PhD

Dr. Freedman is Director of the Pancreas Center at Beth Israel Deaconess Medical Center, Chief of the Division of Translational Research, and Professor of Medicine at Harvard Medical School, Boston, Massachusetts. He received his PhD from Yale University School of Medicine in 1981 followed by the MD degree at the University of Connecticut in 1986. He completed his residency and fellowship in Gastroenterology at Beth Israel Hospital and has remained on faculty since 1991. He is an internationally recognized leader in exocrine pancreatic disease with a particular focus on pancreatitis and cystic fibrosis with an extensive research program that encompasses both basic science discovery as well as clinical trials.

Dr. Freedman was recently elected as the Section Vice Chair position of the Pancreatic Disorders (PAN) Section of the AGA Institute Council. With the support of the Cystic Fibrosis Foundation, Dr. Freedman has developed and launched a national initiative to train pediatric and adult gastroenterologists across North America in the diagnosis and treatment of the GI manifestations of CF. Recently, he has extended his training initiatives to now include his recent appointment as Director of the Beth Israel Deaconess Medical Center Internal Medicine Residency Physician Scientist Track.

Linda P. Fried, MD, MPH

Dr. Fried, Dean of the Mailman School of Public Health is a public health leader in the fields of epidemiology and geriatrics. She has dedicated her career to the science of healthy aging and defining how to transition to a world where greater longevity benefits people of all ages. An internationally renowned scientist, she has done seminal work in defining frailty as a clinical syndrome and illuminating both its causes and the potential for prevention as keys to optimizing health for older adults. Her scientific discoveries have transformed medical care and public health globally, and our understanding of how to build successful societies of longer lives. Under Fried's visionary leadership, the Mailman School continues to be a leader in transforming the health of populations and is one of the top five NIH-funded schools of public health. Fried led the School to build the nation's first program on climate and health and a multidisciplinary program that delivers economic evidence on the value of prevention. Fried opened the Columbia Center for Aging and elevated Columbia's leadership role in research, policy and programming to support healthy cities. As a leader in public health education, she initiated Columbia/Mailman's innovative interdisciplinary public health curriculum that emphasizes a life-course approach to prevention of disease and disability. Fried is an elected member of the U.S. National Academy of Medicine, as well as the President of the Association of American Physicians, the elected society of the U.S. leading physician scientists. She is the first Dean of a School of Public Health to be President of AAP. She is also Co-Chair of the World Economic Forum's Global Council on the Future of Human Enhancement and on the Steering Committee for their Council on Human Centric Health.

Julie Gerberding, MD, MPH

Dr. Gerberding is Executive Vice President and Chief Patient Officer, Strategic Communications, Global Public Policy, and Population Health at Merck & Co., Inc., where she also has responsibility for the "Merck for Mothers" global program to prevent maternal mortality and the Merck Foundation. She joined Merck in January 2010 as president of Merck Vaccines and led efforts to make the company's vaccines more available and affordable to people in resource-limited countries around the world.

She left her tenured faculty position at the University of California, San Francisco in 1998 to lead the U.S. Centers for Disease Control and Prevention (CDC) Division of Healthcare Quality Promotion and then served as the CDC Director from 2002 to 2009.

Dr. Gerberding has received more than 50 awards and honors, including the United States Department of Health and Human Services (DHHS) Distinguished Service Award for her leadership in responses to anthrax bioterrorism and the September 11, 2001 attacks. She was named to Forbes Magazine's 100 Most Powerful Women in the World in 2005 through 2008 and to TIME Magazine's 100 Most Influential People in the World in 2004.

Speaker Biographies

Gary H. Gibbons, MD

Dr. Gibbons is Director of the National Heart, Lung, and Blood Institute (NHLBI) at the National Institutes of Health (NIH), where he oversees the third largest institute, with an annual budget of approximately $3 billion and nearly 1,000 employees.

Since being named Director of the NHLBI, Dr. Gibbons has enhanced the investment in fundamental discovery science by steadily increasing the payline and number of awards for early and established investigators. He also provides leadership to advance several key NIH initiatives such as precision medicine and biomedical research workforce diversity.

Dr. Gibbons has made many scientific contributions and received several patents for innovations in the fields of vascular biology, genomic medicine, and the pathogenesis of vascular diseases. His research focuses on investigating the relationships between clinical phenotypes, behavior, molecular interactions, and social determinants on gene expression and their contribution to cardiovascular disease.

Prior to his current position, Dr. Gibbons was a member of the faculty at Stanford University from 1990-1996 and Harvard Medical School from 1996-1999. He joined Morehouse School of Medicine in 1999, where he served as the founding director of the Cardiovascular Research Institute, Chair of the Department of Physiology, and Professor of Physiology and Medicine.

Dr. Gibbons is an elected member of the Institute of Medicine of the National Academies of Sciences, a Robert Wood Johnson Foundation Minority Faculty Development Awardee, a Pew Foundation Biomedical Scholar, and an Established Investigator of the American Heart Association.

Laurie H. Glimcher, MD

Dr. Glimcher is President and CEO of Dana-Farber Cancer Institute, Director of the DF/HCC and the Richard and Susan Smith Professor of Medicine. Previously, she held positions at Weill Cornell Medical College and Harvard Medical School. Dr. Glimcher speaks nationally and internationally on rheumatology, cancer, immunology, skeletal biology and translational medicine.

Joel F. Habener, MD

Dr. Habener received his B.S. degree Cum Laude in 1960 from the University of Redlands, Redlands, California and in 1965 his M.D degree from the University of California School of Medicine, Los Angeles, California. Dr. Habener is Professor of Medicine at the Harvard Medical School, Associate Physician at the Massachusetts General Hospital, and a former Investigator with the Howard Hughes Medical Institute. He is the Director of the Laboratory of Molecular Endocrinology in the Department of Medicine at the Massachusetts General Hospital. His research interests are in the fields of obesity, diabetes, and metabolism with a focus on the interactions of growth factors and morphogens on the expression of transcription factors during development and in the regulation of hormone production by endocrine organs of the body. He has authored over 450 research articles, books and reviews on these subjects. He is a discoverer of the insulinotropic hormone glucagon-like peptide-1, cyclic AMP response element binding protein, the islet duodenal homeodomain protein, and the existence of multipotent stem cells in the pancreas. Dr. Habener holds several patents on these discoveries. Dr. Habener's discoveries contributed to the development of glucagon-like peptide-1, currently on the market as a treatment for diabetes. Dr. Habener is a member of several editorial boards of scientific journals and has served on many advisory committees of pharmaceutical companies and the National Institutes of Health. He is the recipient of several awards, including the Robert H. Williams Distinguished Leadership Award by the Endocrine Society in 1999.

Aida Habtezion, MD, MSc

The Habtezion lab aims to understand immune mechanisms and identify potential immune-based therapeutic targets for pancreatitis and inflammatory bowel disease. The lab studies leukocyte trafficking and immune responses pertaining to the gastrointestinal organs in states of both health and disease. The lab demonstrated a beneficial role and mechanism for the stress inducible anti-inflammatory enzyme, hemeoxygenase-1, and its downstream effectors in acute pancreatitis. In chronic pancreatitis the lab demonstrated macrophage-pancreas stellate cell crosstalk that contributes to disease progression and fibrosis. The significance of this crosstalk is further demonstrated by targeting macrophage polarization and function, as well as altering disease course in established experimental disease. More recently the lab showed a mechanism via which environmental factors such as smoking (independent risk factor for the development of chronic pancreatitis and pancreatic cancer) promote immune and pancreas stellate cell interaction leading to progression of chronic pancreatitis. The lab is currently working to elucidate targetable immune pathways that alter and/or reverse the course of disease. A second major project in the lab pertains to understanding immune responses in the intestine and in inflammatory bowel disease (IBD). The lab is currently trying to understand disease heterogeneity among IBD patients using immune profiling and approaches that determine host immune-microbiome interactions. In addition, using experimental models, the lab is actively pursuing immune-enteric nervous system interaction and intestine-specific leukocyte recruitment in order to develop intestine specific therapeutic targets to ameliorate disease.

Speaker Biographies

Jens J. Holst, MD, DMSc

Dr. Holst is professor of Medical Physiology in the Department of Biomedical Sciences at the Faculty of Health Sciences, University of Copenhagen, where he is also the vice-chairman. Since 2006 he has been director of the Research Cluster for Diabetes and Obesity at the Faculty, and, since 2010, Scientific Director at section for Translational Metabolic Physiology, Novo Nordisk Foundation Center for Basic Metabolic Research. Dr. Holst received his medical degree from the University of Copenhagen in 1970 and the Doctor of Medical Sciences degree in 1978. His scientific work has been focused on the regulatory peptides of the pancreas and the gut and their importance in the regulation of the functions of the GI-tract and metabolism, with particular focus on blood glucose and appetite regulation, obesity and diabetes. A particular emphasis has been on the role of the incretin hormones of the gut (GLP-1 and GIP). Dr. Holst's scientific achievements include the discovery of GLP-1 (glucagon-like peptide 1) as the gut hormone being responsible for the glucose-induced gastrointestinal stimulation of insulin secretion and his subsequent both basic and translational research in this field. He is a member of several distinguished academic organizations, including the Danish Academy for Natural Sciences and the Royal Danish Academy of Science and Letters, and has been the recipient of the Anders Jahre Award for Medical Research, the Odd Fellow Award for Medical Research the Paul Langerhans Medal of the German Diabetes Association and the Claude Bernard Award of the European Society for the Study of Diabetes.

Eric R. Kandel, MD

Dr. Kandel is University Professor at Columbia University; Kavli Professor and Director, Kavli Institute for Brain Science; Co-Director, Mortimer B. Zuckerman Mind Brain Behavior Institute; and HHMI Investigator. A graduate of Harvard College and N.Y.U. School of Medicine, Kandel trained in Neurobiology at the NIH and in Psychiatry at Harvard Medical School. He joined the faculty of the College of Physicians and Surgeons at Columbia University in 1974 as the founding director of the Center for Neurobiology and Behavior.

Dr. Kandel's research has been concerned with the molecular mechanisms of memory storage in *Aplysia* and mice. More recently, he has studied animal models in mice, age related memory disorders, post-traumatic stress disorders, nicotine, alcohol, marijuana and cocaine addiction.

Dr. Kandel has received twenty-three honorary degrees, is a member of the U.S. National Academy of Sciences as well as being a Foreign Member of the Royal Society of London and a member of the National Science Academies of Austria, France, Germany and Greece. He has been recognized with the Albert Lasker Award, the Wolf Prize of Israel, the National Medal of Science USA and the Nobel Prize for Physiology or Medicine in 2000.

Mariana Kaplan, MD

Mariana Kaplan is Senior Investigator and Chief of the Systemic Autoimmunity Branch at NIAMS/NIH. Dr. Kaplan's research has focused on identifying mechanisms of organ damage and premature vascular disease in systemic autoimmunity. More specifically, she investigates how innate immunity (in particular, type I interferons and myeloid cells) promote end-organ damage in systemic lupus erythematosus, rheumatoid arthritis and other systemic autoimmune diseases. Recently, her research has focused on identifying abnormalities of neutrophil subsets and the role of neutrophil extracellular traps (NETs) in lupus, vasculitis and rheumatoid arthritis, both of which may contribute to the development of autoimmune responses and to end-organ damage. Dr. Kaplan also has an interest in identifying novel therapeutic targets that may prevent premature vascular damage in systemic autoimmunity, as well as the role of environmental triggers in the induction of autoimmunity. Moreover, she has led clinical trials to identify mechanisms that reduce blood vessel dysfunction in autoimmune and chronic inflammatory disorders. In addition to her research activities, Dr. Kaplan is an active clinician and teacher and is involved in the development of various clinical trials for patients with autoimmune diseases at the NIH Clinical Center. She has served in various roles at the American College of Rheumatology/ Rheumatology Research Foundation, the American Association of Immunologists, the Journal of Immunology, and the Lupus Foundation of America. She was elected to the American Society for Clinical Investigation, and received the Henry Kunkel Young Investigator Award and the Edmund L. Dubois Memorial Lectureship, both from the American College of Rheumatology. Dr. Kaplan received the 2015 Evelyn V. Hess Award from the Lupus Foundation of America in recognition of her significant contributions to lupus research, diagnosis, and treatment and the Charles L. Christian Award for advances in understanding lupus in 2016.

Robert J. Lefkowitz, MD

Dr. Lefkowitz is James B. Duke Professor of Medicine and Professor of Biochemistry and Chemistry at the Duke University Medical Center. He has been an Investigator of the Howard Hughes Medical Institute since 1976. Dr. Lefkowitz began his research career in the late 1960's and early 1970's, when there was no clear consensus that receptors even existed. His group spent 15 years developing techniques for radioligand binding, solubilization, purification, and reconstitution of the four adrenergic receptors known at the time. In 1986, Dr. Lefkowitz transformed the understanding of what had become known as G protein coupled receptors (GPCRs), when he and his colleagues cloned the gene and cDNA for the β_2 adrenergic receptor, and recognized its sequence homology with rhodopsin, thus establishing them as the first members of a new family of proteins, the Seven Transmembrane Receptors (7TMRs). This superfamily is now known to be the largest, most diverse, and most therapeutically accessible. Dr. Lefkowitz's lab also discovered and cloned the G protein coupled receptor kinases (GRKs) and β-arrestins which mediate receptor desensitization and discovered "biased" signaling through β-arrestins or G proteins. Most recently, he has been applying the tools of structural biology to understand biased signaling at atomic level resolution. He has received numerous awards and honors, including the National Medal of Science, the Shaw Prize, the Albany Prize, and the 2012 Nobel Prize in Chemistry. He was elected to the USA National Academy of Sciences in 1988, the Institute of Medicine in 1994, and the American Academy of Arts and Sciences in 1988.

Speaker Biographies

W. Ian Lipkin, MD

Dr. Lipkin the John Snow Professor of Epidemiology and Professor of Neurology and Pathology at Columbia University is internationally recognized for the development of genetic methods for microbial surveillance and discovery. He directs the Center for Infection and Immunity at Columbia University and the NIH Center for Diagnostics and Discovery. His contributions include the first use of genetic methods to identify an infectious agent; implication of West Nile virus as the cause of the encephalitis in North America in 1999; invention of MassTag PCR and the first panmicrobial microarray; first use of deep sequencing in pathogen discovery; and molecular characterization of more than 800 viruses. He has been active in translating science to the public through print and digital media and was chief scientific consultant for the Soderbergh film, Contagion. His honors include Pew Scholar in the Biomedical Sciences, Kinyoun Lecturer National Institutes of Health, Oxford University Simonyi lecturer, the Mendel Medal, Bernard Fields lecturer and service on the Advisory Committee to the Director of the NIH. He is a fellow of the American Society for Microbiology, American Association for the Advancement of Science, Royal Geographic Society, Wildlife Conservation Society and the Infectious Diseases Society of America, and is a Member of the Association of American Physicians. In 2016 he received the International Science and Technology Cooperation Award, the top science honor in China for his contributions to the advancement of science in the country.

Sarah Hollingsworth Lisanby, MD

Dr. Hollingsworth Lisanby is the director of Division of Translational Research at the National Institute of Mental Health (NIMH). As director for the Division of Translational Research, Dr. Lisanby oversees a research funding portfolio of approximately $400 million and helps set a national agenda for research on mental illness. She also works with Dr. Carlos Zarate and colleagues in the Division of Intramural Research Programs as Director of the Noninvasive Neuromodulation Unit (NNU), creating an important bridge between the Institute's extramural and intramural research efforts.

Dr. Lisanby is one of the leading researchers in the area of neuromodulatory interventions for treating major depression, serving as a principal investigator on studies that range from basic research through clinical trials. Additionally, she is a prolific author with approximately 200 scientific articles and book chapters, and she has also received national and international recognition.

Dr. Lisanby's prodigious research life has been matched by extensive service to NIMH and beyond. She has been a member of the NIMH Board of Scientific Counselors since 2013, and has chaired or been a member of a variety of NIH Study Sections since 2004. Dr. Lisanby also serves on the FDA Neurological Devices Advisory Panel, is on five editorial boards, and has held key leadership positions with numerous professional associations, including Chair of the American Psychiatric Association Task Force to Revise the Practice on Electroconvulsive Therapy (ECT).

Hailing from Arlington, VA, Dr. Lisanby received dual Bachelor of Science degrees in Mathematics and Psychology from Duke University in 1987, where she went on to receive an MD and complete a residency in Psychiatry, serving as Chief Resident. In 1995 Dr. Lisanby joined Columbia University for a postdoctoral fellowship, and became an Assistant Professor in 1998. She was named Director of the Division for Brain Stimulation and Neuromodulation at Columbia University/New York State Psychiatric Institute in 2005, and Professor of Clinical Psychiatry at Columbia University in 2007, before returning to her alma mater as Chair for the Department of Psychiatry at Duke University in 2010, where she served for 5 years as tenured professor, department chair, and founding director of the Brain Stimulation & Neurophysiology Division.

David Meltzer, MD, PhD

Dr. Meltzer is Chief of the Section of Hospital Medicine, Director of the Center for Health and the Social Sciences and the UChicago Urban Health Lab, and Chair of the Committee on Clinical and Translational Science at The University of Chicago, where he is The Fanny L. Pritzker Professor in the Department of Medicine, the Harris School of Public Policy Studies and the Department of Economics. Meltzer's research explores problems in health economics and public policy with a focus on the theoretical foundations of medical cost-effectiveness analysis and the cost and quality of hospital care. He currently leads a CMMI Challenge award to study the effects of improved continuity in the doctor-patient relationship between the inpatient and outpatient setting on the costs and outcomes of care for frequently hospitalized Medicare patients. Meltzer completed his MD and PhD in economics at the University of Chicago and his residency in internal medicine at Brigham and Women's Hospital. His awards include the Garfield Award from Research America, the AHRQ Eisenberg Excellence in Mentoring Award, and the AAMC Learning Healthcare System Award. He is a member of the National Academy of Medicine.

Sanjay Misra, MD, FAHA, FSIR

Dr. Misra received his Bachelor's degree in Electrical Engineering from Drexel University. After completing his Medical Degree from Hahnemann University School of Medicine in Philadelphia, Pennsylvania, Dr. Misra completed an Internship and Residency where he was Chief Resident, Diagnostic Radiology. He completed a 2-year fellowship in Cardiovascular and Interventional Radiology at The Johns Hopkins Hospital in Baltimore, Maryland. Dr. Misra is Board Certified in Diagnostic Radiology with additional Certification in Vascular Intervention.

He is currently Professor and Chair of the Vascular Radiology Research Department at Mayo Clinic where he has been faculty since July 2000. He has published more than 138 peer-reviewed manuscripts of which greater than 45 are in the vascular biology field. He has been Program Director of the VIR Fellowship and been involved with the mentorship of more than 40 Radiology residents and post-doctoral fellows in his lab.

As a physician scientist, his laboratory studies the cellular signaling and mechanisms responsible for reducing venous neointimal hyperplasia and treating renal ischemic injury using small molecule inhibitors, nano-therapies, anti-angiogenic therapies, and cell based approaches. His clinical interests include treatment of patients with peripheral arterial disease, renal vascular disease, and hemodialysis vascular access.

Speaker Biographies

Roberta B. Ness, MD, MPH

Dr. Ness has often been called one of America's foremost experts in innovative thinking. She is author of *Innovation Generation,* a systematic method for how anyone can become more innovative, and an accompanying workbook of innovation training exercises, *Creativity in the Sciences.* Her book *Genius Unmasked*, the story of genius scientists of the 20th century and how they thought, has been popularly acclaimed. Her fourth book, *The Creativity Crisis*, focuses on how organizations can foster creative thinkers (all published by Oxford University Press). Dr. Ness is a popular speaker. Over the past 3-4 years, she has given over 90 talks and workshops on innovative thinking at America's top universities, science and technology professional societies, and R&D intensive corporations. She teaches a class on the topic at the University of Texas and has a funded grant training graduate students to apply creativity to the sciences. A recognized expert in medicine and public health, Dr. Ness recently stepped down as dean of The University of Texas School of Public Health, one of the largest such Schools in the nation. Dr. Ness holds the Rockwell Endowed Professorship in Public Health and is Vice President for Innovation. She is a tenured professor of medicine, obstetrics/gynecology, and epidemiology. Dr. Ness firmly believes, and has the data to prove that today's global workforce can be taught to maximize their human potential through more innovative thinking. Her insights as a scientist and administrator have put her in a unique position to imagine and test organizational techniques to craft creative organizations.

M. Kerry O'Banion, MD, PhD

Dr. O'Banion, Professor and Vice-Chair of Neuroscience and Professor of Neurology at the University of Rochester School of Medicine & Dentistry, received a BS in Biology in 1980 and MD and PhD in 1987 from the University of Illinois at Urbana-Champaign, being named to the AOA National Medical Honor Society. Dr. O'Banion has devoted the past 25 years to understanding the role of neuroinflammation in acute and degenerative brain disease and is an international leader in the fields of Alzheimer's disease and CNS radiation injury. His original discovery that cyclooxygenase-2 plays a major role in inflammation fueled the development of several important new drugs, the COX 2 inhibitors (e.g. Celebrex®).

Dr. O'Banion oversees laboratory courses in neuroanatomy for graduate and medical students, co-directs a course on Neuroinflammation, and presents lectures for a NASA sponsored summer school at Brookhaven National Laboratories. Dr. O'Banion also directs the Medical Scientist Training Program (MSTP) at the University of Rochester, a position held since 2000. Dr. O'Banion was Chair of the MD-PhD Section of the Association of American Medical Colleges' Graduate Research, Education, and Training (GREAT) Group in 2009-2010, and currently serves on the American Physician Scientists Association (APSA) Board of Directors.

David Ostrow, MD, PhD

Dr. Ostrow co-founded the first gay community health center, now the Howard Brown Health Center of Chicago during his training in the MD/PhD Program at the University of Chicago. There he identified Hepatitis B as a common sexually transmitted infection (STI) among gay men, which led to his Chicago PI role in the CDC-funded Hep B Epidemiology and Vaccine Efficacy Studies (1976-81). As a PI, his research has focused on the relationship between sexual behavior, drug use and the evolution of HIV transmission and prevention among drug using MSM. He has published 175 peer-reviewed papers, 50+ book chapters and presented 60+ times at International AIDS Conferences. He is an Investigator or consultant on most of the NIDA funded studies of drugs, alcohol and HIV in the MACS, including his own study of the Social and Risk Networks Assessment of younger Black MSM (2009-2011).

Since the late '90s, Dr. Ostrow has been active in the movement to change failed national drug policies, such as cannabis prohibition, with more effective and compassionate policies.

Neelroop (Neel) Parikshak, MD, PhD

Dr. Parikshak was born in Ahmedabad, India. While he was in elementary school, his family moved throughout the midwest and ultimately settled in West Virginia. Neel first became interested in the neurosciences around the end of high school, when he experienced a few months of facial paralysis from Bells Palsy. He next attended college at Rice University, where he majored in Mathematics and Biochemistry and found that he enjoyed both neuroscience and medicine. He then joined the MD/PhD program at UCLA and completed his thesis work in the laboratory of Daniel H. Geschwind. His work focused on the genomics of neuropsychiatric disorders, with a particular emphasis on genes and pathways affected in autism spectrum disorder. He is generally interested in applying genomics, bioinformatics, and molecular biology approaches to dissect the etiology of complex diseases and identify data-driven therapeutic targets. He will be starting a Neurology residency program this summer and plans to continue pursuing his research interests. If all goes as planned, he hopes to be juggling clinical work, running a lab, and having a happy family life at home before he gets too many more grey hairs.

Peter C. Preusch, PhD

Dr. Preusch is Acting Director of the Division of Cell Biology and Biophysics, NIGMS, NIH, DHHS. He has been with the NIH since 1990, serving first as a review administrator in DRG (CSR), then as a program director and later as the biophysics branch chief at NIGMS. He has managed fellowship and training grants in the Pharmacological Sciences, Cell and Molecular Biology, and the Medical Scientist Training Program; and in 2006 served as the Acting NIH Training Officer in the Office of the Director, NIH. He helped launch the K9/R00 grant program and has contributed to implementing the recommendations of the Physician Scientists Workforce Working Group of the Advisory Committee to the Director, NIH. Most recently he has been leading implementation of the Maximizing Investigators' Research Award (MIRA) program.

He received his B.S. in biochemistry from Pennsylvania State University in 1974 and his PhD in biochemistry with a minor in chemical engineering from Cornell University in 1979. He was a postdoctoral fellow at the University of Wisconsin from 1979 to 1983. From 1983 to 1990, he was Assistant Professor of Chemistry at the University of Akron.

Speaker Biographies

Christian P. Schaaf, MD, PhD

Dr. Schaaf is an assistant professor in the Department of Molecular and Human Genetics at Baylor College of Medicine and an investigator at the Jan and Dan Duncan Neurological Research Institute at Texas Children's Hospital. He studies the genetic causes of neurodevelopmental and neuropsychiatric disorders. His work led to the discovery of multiple new disease genes, and two disorders have been named after him: Schaaf-Yang syndrome and Bosch-Boonstra-Schaaf Optic Atrophy syndrome.

Dr. Schaaf groundbreaking work has been recognized with many awards, including a Doris Duke Clinical Scientist Development Award, the William K. Bowes Award for Medical Genetics by Partners Healthcare and Harvard Medical School, and the inaugural ASCI Seldin~Smith Award for Pioneering Research.

He has authored four books, including a major textbook of medical genetics that has been translated into several languages. He is a passionate teacher and educator, currently serving as the Chair of Education for the American College of Medical Genetics. Most importantly, he is an advocate for his patients. He empowers families by helping them understand what human genetics has to offer in patient care, management, counseling, and family planning. Together with family support organizations, Dr. Schaaf works tirelessly towards care plans and therapeutic opportunities that will improve the quality of life of those affected with neuropsychiatric disease.

Amita Sehgal, PhD

Dr. Sehgal is the John Herr Musser Professor of Neuroscience, Investigator of the Howard Hughes Medical Institute and Director of the Chronobiology Program at the University of Pennsylvania. Prof. Sehgal received her PhD from the Weill Graduate School of Cornell University and conducted her postdoctoral work at Rockefeller University. Her research focuses on the genetic basis of circadian rhythms and sleep, using primarily Drosophila as a model system. Sehgal's contributions to the circadian field include the identification of circadian genes, elucidation of mechanisms of the circadian clock, the first report of a mechanism that resets animal clocks to light and dissection of pathways that transmit time-of-day signals from the clock to produce rhythmic behavior and physiology. To understand the mechanisms underlying sleep homeostasis, Sehgal and her colleagues developed a Drosophila model for sleep, and since then have discovered sleep-regulating genes, identified sleep circuits in the fly brain and also a function for sleep in early life. Sehgal serves on national and international advisory panels and also as editor for several journals. Her work has been recognized through a number of awards and honors, which include the Outstanding Scientific Acievement award from the Sleep Research Society and the Ellison Senior Scholar award. Sehgal is an elected member of the National Academy of Medicine, the American Academy of Arts and Sciences and the National Academy of Sciences USA.

Karen Sibert, MD

Dr. Sibert was born and raised in Amarillo, Texas in the 1960s and accepted to Princeton as part of the school's second entering class of female students. She graduated with an English degree and became a reporter for the Wall Street Journal. She later applied and was accepted to Baylor College of Medicine. After choosing to specialize in anesthesiology, Karen completed her residency training and fellowship in anesthesiology at the Yale University Hospital in New Haven, Connecticut. She then joined the faculty at Duke University School of Medicine. Since 1999, Karen has worked in Los Angeles as a clinical anesthesiologist and an Associate Professor of Anesthesiology, teaching residents and fellows in training. She hasn't let that get in the way of her writing career. Besides authoring aPennedPoint blog, Karen was recently published in The New York Times and is a regular contributor to the CSA Bulletin. Karen's name also frequently appears as an author of articles in medical journals and chapters in both medical and general interest books. She practices full time, specializing in anesthesia for thoracic surgery and other high-risk adult cases.

Solomon Snyder, MD, DSc, DPhil

Dr. Snyder was born and raised in Washington, D.C. and received his undergraduate and medical training at Georgetown University; Research Associate training with Julius Axelrod at the NIH; and psychiatric training at the Johns Hopkins Hospital. He joined the faculty of the Johns Hopkins University School of Medicine (Asst Professor Pharmacology, 1966-1968; Associate Professor Pharmacology/Psychiatry, 1968-1970; Professor, 1970). In 1980 he established the Department of Neuroscience and served as Director (1980-2006). He is presently Distinguished Service Professor of Neuroscience, Pharmacology and Psychiatry.

Dr. Snyder is the recipient of numerous professional honors, including the Albert Lasker Award for Basic Biomedical Research (1978), the National Medal of Science (2005), the Albany Medical Prize (2007), the Warren Alpert Prize, Harvard University (2014), numerous Honorary Doctor of Science degrees, and the Salk Institute Science Award (2016). He is a member of the United States National Academy of Sciences and a Fellow of the American Academy of Arts and Sciences and the American Philosophical Society. He is the author of more than 1000 journal articles and several books including Drugs and the Brain (1986), and Brainstorming (1989).

Speaker Biographies

Eric Svensson, MD, PhD

Dr. Svensson is a physician scientist who is currently a director at the Novartis Institutes of Biomedical Research (NIBR) based in Cambridge, MA. Dr. Svensson obtained his MD/PhD degree from the UCLA School of Medicine. His PhD work there focused on investigating the regulation of protein glycosylation. He moved to the University of Chicago for his internal medicine residency training, cardiology fellowship, and a post-doctoral research fellowship in cardiovascular gene therapy and cardiac development. He subsequently became a faculty member in the Section of Cardiology at the University of Chicago where he practiced clinical cardiology, specializing in the care of adults with congenital heart disease. He also ran a basic research laboratory focused on elucidating the molecular mechanisms regulating cardiac development. While at the University of Chicago, Dr. Svensson established and directed the Physician Scientist Development Program, a program designed to provide further clinical and research training to graduates of MD/PhD programs.

Dr. Svensson joined the Translational Medicine department in NIBR in late 2012. At NIBR, he works with teams of basic researchers and clinical scientists to transition promising cardiovascular therapeutics from the lab into phase 1 and phase 2 clinical trials.

Chris Williams, MD, PhD

Dr. Williams is the Associate Dean for Physician-Scientist Education and Training and Associate Professor of Medicine, Gastroenterology, and Cancer Biology, and Staff Gastroenterologist at the Nashville VAMC, and the current director of both the Medical Scientist & Physician-Scientist Training Programs at Vanderbilt University. His clinical interests are in the management of inflammatory bowel disease and the prevention of IBD related complications, specifically colitis-associate carcinoma. His research program is focused on intestinal injury response/repair programs with the goal of understanding how these epithelial integrity programs are perturbed in the pathogenesis of IBD and more importantly in the progression to malignancy with the overarching goal of identifying novel therapeutic targets or biomarkers in these disease. Dr. Williams received his undergraduate degree from Brigham Young University before matriculating to Vanderbilt University as an MSTP student. He was selected for the ABIM Research Pathway Residency and received sub-specialty training in Gastroenterology. He joined the Vanderbilt faculty in 2007. His research program has been supported by the American Cancer Society, the VICC Young Ambassadors, the NIH (NIDDK), the Veterans Administration, and the Crohn's and Colitis Foundation of America (CCFA). He is currently involved in MSTP/PSTP integration strategies with the AAMC and AAIM.

Myles Wolf, MD, MMSc

Dr. Wolf is Professor of Medicine and Chief of the Division of Nephrology at the Duke University School of Medicine. Dr. Wolf attended medical school at the State University of New York Downstate, and completed his training in internal medicine and nephrology at the Massachusetts General Hospital. Dr. Wolf conducts epidemiological, clinical and basic research of fibroblast growth factor 23 (FGF23) and its role in disordered mineral metabolism across the spectrum of chronic kidney disease. In recognition of his work on FGF23, Dr. Wolf was elected to the American Society of Clinical Investigation in 2010.

Kristine Yaffe, MD

Dr. Yaffe attended Yale University for her undergraduate degree, received her medical degree at the University of Pennsylvania, and completed residencies in Neurology and Psychiatry at the University of California, San Francisco. She is the Roy and Marie Scola Endowed Chair, Vice Chair of Research in Psychiatry, and Professor of Psychiatry, Neurology and Epidemiology at UCSF. She is also the Chief of NeuroPsychiatry and Director of the Memory Evaluation Clinic at the San Francisco Veterans Affairs Medical Center. In both her research, clinical work, and mentoring, she has directed her efforts towards improving the care of patients with cognitive disorders and other geriatric neuropsychiatric conditions.

Dr. Yaffe's research focuses on the epidemiology of cognitive aging. As the principal investigator of multiple grants from the NIH, Department of Defense, and other foundations, she is a leading expert in the modifiable risk factors of dementia, and she has published over 400 peer-reviewed articles (H-index=106) in numerous prestigious journals including the Lancet, BMJ, JAMA, and NEJM. Dr. Yaffe served as the Co-Chair of the Institute of Medicine's Committee on Cognitive Aging which released a report last year entitled, "Cognitive Aging: Progress in Understanding and Opportunities for Action". She is also a member of the Council of the German Center for Neurodegenerative Diseases and the Alzheimer's Association Medical & Scientific Advisory Council. Dr. Yaffe has been recognized by Thomas Reuters as one of the World's Most Influential Scientific Minds and has received several national awards for her distinguished, scholarly work.

Tadataka (Tachi) Yamada, MD

Dr. Yamada is a Venture Partner at Frazier Healthcare Partners. He was previously Executive Vice-President, Chief Medical & Scientific Officer and a Board Member of Takeda Pharmaceuticals. He joined Takeda after serving as President of the Bill & Melinda Gates Foundation Global Health Program where he managed $9 billion in programs directed at addressing major health challenges of the developing world. Previously, he had served as Chairman, Research and Development and a Member of the Board of GlaxoSmithKline and before that as Chair of the Department of Internal Medicine and Physician-in-Chief at the University of Michigan Medical Center.

Dr. Yamada holds a bachelor's degree in history from Stanford University and obtained his MD from New York University School of Medicine. In recognition of his contributions to medicine and science he has been elected to membership in the National Academy of Medicine (US), the Academy of Medical Sciences (UK) and the National Academy of Medicine (Mexico) and he has received an honorary appointment as Knight Commander of the Most Excellent Order of the British Empire (KBE). He is a Past-President of the Association of American Physicians and of the American Gastroenterological Association and he has served as a member of the President's Council of Advisors on Science and Technology. He is currently Vice-Chair of the Council of the National Academy of Medicine and serves on the Board of Directors of the Clinton Health Access Initiative.

Leonard I. Zon, MD

Dr. Zon is the Grousbeck Professor of Pediatric Medicine at Harvard Medical School, an Investigator at Howard Hughes Medical Institute, and the Director of the Stem Cell Program at Boston Children's Hospital. He is internationally-recognized for his pioneering work in stem cell biology and cancer genetics, and has been the preeminent figure in establishing zebrafish as an invaluable genetic model for the study of blood and hematopoietic development.

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1

Loss of Function Mutations in *GALNT14* Predispose to IgA Nephropathy

***S Prakash*[1], K Kiryluk[2], Y Li[2], D Fasel[2], N Steers[2], N Papeta[2], S Shapiro[2], J Novak[3], B Julian[3], M Renfrow[3], H Snyder[2], R Lifton[4], S Sanna-Cerchi[2], A Gharavi[2]**

[1]Rutgers University - New Jersey Medical School, New York, NY; [2]Columbia University, New York, NY; [3]University of Alabama, Birmingham, AL; [4]Yale University, New Haven, CT

IgA Nephropathy (IgAN) is the most commonly diagnosed primary glomerulonephritis and a major cause of kidney failure worldwide. A fundamental pathogenic defect in IgAN patients is the production of aberrantly O-glycosylated IgA1, which acts as an auto-antigen resulting in the generation of immune complexes that deposit in the kidney, causing injury. Genome wide association studies have identified common variants for IgAN that play a role in mucosal immunity, but no association with glycosyltransferases known to participate in IgA1 O-glycosylation has been detected. Although familial aggregation has been reported, it is not known whether rare variants contribute to the pathogenesis of IgAN. We combined genome wide linkage analysis and whole exome sequencing in an IgAN family with 6 affected individuals. Systematic annotation of rare variants within shared intervals led to the identification of a segregating mutation (p. R315X, aa 552) in *GALNT14,* encoding a glycosyltransferase that transfers N-acetylgalactosamine to hydroxyl groups on serine and threonine residues. Loss of function (LoF) variants in *GALNT14* are found at a frequency of 0.046% of >140,000 individuals in the gnomAD database. Interestingly, GALNT14 localizes to the germinal center of human lymph nodes and the white pulp of human and murine spleen, and is also highly expressed in human IgA and IgG producing B cells. In the index family, mutation carriers had significantly increased levels of total IgA and reduced fraction of galactose deficient IgA1, indicative of a role in aberrant IgA1 O-glycosylation. To replicate findings, we studied independent cohorts of cases and controls and identified 4 rare, heterozygous *GALNT14* LoF variants in 462 IgAN cases and 3 LoF variants in 3503 ethnicity-matched controls (0.43% vs. 0.042%, OR= 12.9, p= 2 x 10^{-3}). To further follow-up these findings, we studied *Galnt14* null mice. *Galnt14* null mice are viable and fertile without any apparent pathology, but after 8 months of age, they display spontaneous glomerular deposition of IgA, without a concomitant increase in serum IgA levels. As most IgA is produced in the intestinal mucosa and IgAN is often triggered by mucosal infection or inflammation, we induced experimental colitis with dextran sodium sulfate (DSS) in 3-month old *Galnt14* null mice. Induction of DSS colitis resulted in increased glomerular IgA deposition in *Galnt14* null mice without a detectable increase in serum IgA levels. In conclusion, rare LoF variants in *GALNT14* are detected in nearly 1% of IgAN patients and impart a large effect on susceptibility to disease. Our data also identifies a novel role for *GALNT14* in IgA O-glycosylation, suggesting that it participates in IgAN pathogenesis by the dysregulation of IgA production during mucosal inflammation.

2

Modification of LPS by EptB Inhibits Intelectin Binding and Increases Systemic Inflammation During *Salmonella* Infection

***L Zhang*, F Rivera-Chavez, H Hiyoshi, A Baumler**

University of California, Davis School of Medicine, Davis, CA

Salmonella enterica is a highly diverse species of Gram-negative bacteria that can be grouped into typhoidal and non-typhoidal serovars. Non-typhoidal serovars, such as *S.* Typhimurium, cause gastroenteritis and inflammatory diarrhea, whereas typhoidal serovars, such as *S.* Typhi, cause systemic disease with a comparatively decreased inflammatory response. A small percentage of patients infected with *S.* Typhi may become asymptomatic chronic carriers of disease. These individuals serve as reservoirs to transmit infection to others and pose a significant challenge for eradication of typhoid fever. Although *S.* Typhi and *S.* Typhimurium are very closely related organisms, the properties that distinguish the two and allow for the *S.* Typhi carrier state remain poorly understood. Previously, comparative analysis of *Salmonella* genomes revealed that typhoidal serovars contain a higher number of pseudogenes than non-typhoidal serovars, suggesting that differences in pseudogene number could play a role in the differential pathogenesis. One such pseudogene in *S.* Typhi is *eptB,* which codes for a phosphoethanolamine transferase that can specifically modify the outer keto-deoxyoctulosonate (KDO) residue of lipopolysaccharide (LPS). Here, we show that loss of *eptB* function in typhoidal serovars may serve as a virulence mechanism that allows *S.* Typhi to evade detection by the immune system, leading to a diminished host inflammatory response and the development of the chronic carrier state. Human intelectin-1 is known to bind to and recognize multiple microbial glycan epitopes, including the KDO of LPS, and may function in detoxification of LPS. Our results demonstrate that LPS isolated from *S.* Typhi, which possesses an *eptB* pseudogene, is bound by intelectin, whereas *S.* Typhimurium LPS is not bound by intelectin. Furthermore, loss of EptB function in *S.* Typhimurium allows binding of intelectin to *S.* Typhimurium LPS. Mice infected with an *eptB* mutant exhibit decreased expression of inflammatory cytokines in the spleen compared to mice infected with the wild type *S.* Typhimurium, suggesting that loss of *eptB* function allows a non-typhoidal *Salmonella* serovar to mimic the stealth phenotype of typhoidal serovars. Together, these results suggest that loss of *eptB* function allows intelectin to bind to and detoxify *Salmonella* LPS, leading to decreased systemic inflammation during infection. These results have broad implications for how pathogens such as *S.* Typhimurium induce systemic shock during infection and may also help to explain a mechanism for how *S.* Typhi is able to evade immune detection and enhance dissemination to systemic sites, leading to the development of the asymptomatic chronic carrier state.

1

Glycomic and Neuroproteomic Alterations in Experimental TBI: Comparative Analysis of Aspirin and Clopidogrel Treatment

***H Abou El Hassan*[1], H Abou Abbass[2], H Bahmad[2], R Zhu[3], S Zhou[3], X Xue[3], E Hamade[4], S Atweh[2], H Darwish[2], K Zibara[4], Y Mechref[3], F Kobeissy[3]**

[1]American University of Beirut, Beirut, Beyrouth; [2]American University of Beirut; [3]Texas Tech University; [4]Lebanese University

The number of patients sustaining traumatic brain injury (TBI) and concomitantly receiving pre-injury antiplatelet therapy such as Aspirin (ASA) and Clopidogrel (CLOP) is on the rise as the population ages. These drugs have been linked with unfavorable clinical outcomes following TBI, where the exact mechanism involved are still unknown. In this novel work, we aim to identify and compare the altered proteome profile imposed by ASA and CLOP when administered alone or in combination, prior to experimental TBI. Furthermore, we assessed differential glycosylation post-translational modification (PTM) patterns following experimental controlled cortical impact (CCI) model of TBI, ASA, CLOP and ASA+CLOP. Ipsilateral cortical brain tissues were harvested 48 hours post injury and were analyzed using an advanced neuroproteomics Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) platform to assess proteomic and glycoproteins alterations. Of interest, differential proteins pertaining to each group (22 in TBI, 41 in TBI+ASA, 44 in TBI+CLOP, and 34 in TBI+ASA+CLOP) were revealed. Advanced bioinformatics, systems biology and clustering analyses were performed to evaluate biological networks and protein interaction maps illustrating molecular pathways involved in the experimental conditions. Results have indicated that proteins involved in neuroprotective cellular pathways were upregulated in the ASA and CLOP groups when given separately. However, ASA+CLOP administration revealed enrichment in biological pathways relevant to inflammation and pro-injury mechanisms. Moreover, results showed differential upregulation of glycoproteins levels in the sialylated N-glycans PTMs which can be implicated in pathological changes. Omics data obtained have provided molecular insights of the underlying mechanisms that can be translated into the clinical bedside setting.

2

Elucidating the Basis for Memory Impairment in Dementia with Lewy Bodies

***D Adamowicz*[1], J Mertens[2], A Paquola[2], D Salmon[3], D Galasko[3], L Hansen[3], E Masliah[3], S Roy[4], F Gage[2]**

[1]University of California, San Diego, San Diego, CA; [2]Salk Institute for Biological Studies, La Jolla, CA; [3]University of California, San Diego, La Jolla, CA; [4]Wisconsin Institute for Medical Research, Madison, WI

Dementia with Lewy bodies (DLB) is a neurodegenerative disease of unknown etiology that shares clinical features with Alzheimer's disease (dementia) and Parkinson's disease (movement disorder). Diagnosis is confirmed at autopsy by the presence of Lewy bodies (intracellular protein aggregates containing α-synuclein) in the brain. When in the brainstem, this Lewy pathology is largely thought to be responsible for movement dysfunction in the disease, but it remains unclear whether memory impairment is related to pathology in the hippocampus. Genetics have also been implicated in DLB in that certain mutations increase the risk of developing the disease. While there are no disease-causing mutations, such as those seen in familial Alzheimer's and Parkinson's, many of the genes implicated in these two conditions can also predispose to DLB. However, the precise nature by which these genetic alterations increase the risk of DLB remains unclear. **Methods:** To address these questions, we pursued two complementary approaches: 1) we examined hippocampal Lewy pathology burden and distribution in 95 neuropathologically and clinically characterized DLB cases from the brain bank of the UCSD Shiley-Marcos Alzheimer's Disease Research Center (ADRC), correlating these pathology data to results from memory testing; 2) we obtained fibroblasts from six clinically diagnosed DLB patients, three unaffected first-degree relatives to use as genetic controls, and four unrelated, non-demented age-matched controls from the same ADRC. We then directly converted these fibroblasts to neurons using existing methods, matured them for several weeks, and used them to investigate differential gene expression and resulting functional differences. **Results:** We found that Lewy pathology in our DLB cases was predominant in two hippocampal-related subregions: the CA2 subfield and the entorhinal cortex (EC). Clinicopathological correlations with measures of verbal and visual memory supported a role for EC Lewy pathology, but not CA2, in causing memory dysfunction. Lewy pathology in CA1—the main output region for CA2—correlated best with results from memory testing despite a milder pathology, suggesting that CA1 may be more functionally relevant than CA2 in the context of memory impairment in DLB. To probe the mechanisms underlying aggregate formation in live neurons, we reprogrammed DLB patient fibroblasts to induced neurons (iNs). The neurons we generated displayed multiple neuronal features, including the expression of neuronal markers, as verified by immunocytochemistry. We conducted RNA-sequencing to compare patient lines and controls, and found that genes related to synaptic function and oxidative stress were differentially regulated. We are now optimizing functional assays based on these findings. **Conclusions:** Hippocampal Lewy pathology in DLB contributes to memory impairment in the disease, as evidenced by our clinicopathological approach. As for how it causes dysfunction, we suspect the direct reprogramming approach using patient-derived iNs will further our understanding of the genetics and molecular mechanisms underlying the disease.

3

Suppression of Inflammasome Activation in Bats

***M Ahn*, A Irving, L Wang**

Duke-NUS Medical School, Singapore

Bats, as the only flying mammals, exhibit several unique features including their long lifespan and hosting many viruses highly lethal to humans like Ebola virus without clinical signs of disease in bats. However, the molecular mechanisms for these observations are totally unknown. Aberrant inflammasome activation leading to excessive systemic inflammation has recently been linked to viral pathogenesis and multiple age-related diseases. Our objective was to characterize the inflammasome activation to gain novel insights into underlying mechanisms of inflammation control in bats. Here we investigated the inflammasome activation in bat primary immune cells and cell lines using ASC speck formation and downstream caspase-1 activation as readouts. Our data show that activation is

significantly reduced in bat cells compared to human counterparts. There is low induction of ASC speck formation and downstream activation in response to both synthetic stimuli and infections with influenza A and MERS virus. Our result so far suggests the involvement of multiple molecular mechanisms at different steps of the signaling pathway. These include unique loss of a sensor gene family at the genomic level and significantly altered expression and function of key inflammasome proteins. These molecular mechanisms of suppressed inflammasome activation in bats can guide the development of new strategies and identifications of novel targets for controlling systemic pathological inflammation.

4

Hijacking Immune Recognition Mechanisms to Rescue Stressed Penumbral Neurons and Inhibit Neuroinflammation after Stroke

***A Alawieh*[1], F Langley[2], S Tomlinson[1]**

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Circulating natural IgM antibodies serve as a danger recognition system of ischemic cells after stroke and promote secondary injury through complement activation. We isolated natural IgM monoclonal antibodies (NIgM) from un-manipulated mice and identified NIgMs that specifically bind ischemic cells. One NIgM, B4IgM which recognizes annexin-IV, reconstituted cerebral ischemia and 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 B4IgM to the complement inhibitor, Crry. We used the fusion construct, B4Crry, as a tool to investigate complement-dependent mechanisms of neuronal loss and recovery after murine stroke, and for characterization as a therapeutic agent. Following a single intravenous injection of B4Crry (8mg/kg) 2-24 hours after murine transient middle cerebral artery occlusion (MCAO), radiolabeled B4Crry targeted specifically to the ischemic brain compared to the contralateral hemisphere and other organs, and resulted in a local and transient inhibition of both IgM binding and complement activation (ELISA, $p < 0.01$) with a tissue retention half-life of 33 hours. Administration of B4Crry up to 24 h after ischemia resulted in sustained neuroprotection throughout the chronic phase (30 days), with significant reductions in cell death and tissue scarring ($p < 0.001$), and significant improvements in neurological deficit scores ($p < 0.01$), forelimb asymmetry (corner task, $p < 0.01$) and cognitive performance (Barnes maze, $p < 0.05$) in adult (12 weeks old) males and females, and in aged (10 months old) mice. To investigate how complement is involved in the rapid loss of neurons after stroke, we used super-resolution immunofluorescence microscopy to assess complement-microglial interactions with stressed neurons in the ischemic penumbra. We show that complement opsonins guide microglial phagocytosis of stressed but salvageable neurons (NeuN+/cfos+), thus reducing the neuronal reserve available to engage in post-stroke recovery and plasticity. Treatment with B4Crry inhibits tagging penumbral neurons for microglial phagocytosis by inhibiting C3d deposition on neurons ($p < 0.01$), while at the same time protecting homeostatic microglial activity and C1q-dependent clearance of apoptotic (caspase3+) cells. Acute inhibition of complement by B4Crry also prevented chronic neurodegeneration that persisted 30 days after stroke by downregulating the expression genes involved in leukocyte chemotaxis, and microglial and complement activation, assessed by Nanostring high-throughput analysis. This resulted in in inhibition of ongoing IgM and complement deposition and inflammatory microgliosis assessed histological analyses. Furthermore, immunostaining of post-mortem brain sections from stroke patients demonstrate that the same neo-epitope recognized by B4Crry in mice is expressed in the ischemic penumbra, but not in the contralateral cortex, of humans ($p < 0.05$). These findings implicate the complement-microglial axis in neuronal loss after stroke, and presents B4Crry as a novel and translational approach to improve motor and cognitive recovery in ischemic stroke patients.

5

Novel Familial NK Cell Immunodeficiency Revealed by Mass Cytometry and Whole Exome Sequencing

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Herpesviruses infect the majority of the human population though few cases result in severe or disseminated infections in immunocompetent patients. Patients with deficiencies in NK cell development or function may suffer from severe, sometimes lethal, infections with DNA viruses such as HSV1 (Herpes Simplex Virus 1). However, few monogeneic causes of functional NK cell disorders have been described to date. Here we describe a case report of a 17-year-old female with a history of severe and frequently recurring HSV1+ gingivostomatitis associated with decreased NK cell function. Clinical testing revealed a novel combination of normal NK cell percentage, perforin/granzyme levels, and CD107 degranulation but severely attenuated cytotoxicity against K562 target cells. Mass cytometry (CyTOF) and whole exome sequencing were used in parallel to investigate these findings. A novel heterozygous mutation in the N-terminal SH2 (nSH2) domain of PLCG2 (G595R) was revealed, correlating to diminished PLCG2 phosphorylation assayed by CyTOF. PLCG2 is a critical signaling enzyme downstream of activating NK cell receptors, the activation of which results in calcium influx and cytolytic granule mobilization. Though mutations in the C-terminal SH2 domain of PLCG2 are associated with the autoinflammatory condition APLAID, mutations in the nSH2 have not been previously investigated as a cause of immunodeficiency. Further investigation revealed cosegregation of reduced NK cell PLCG2 phosphorylation and killing, as well as reduced circulating B cells, in G595R+ family members versus household wildtype controls. Future studies will examine the mechanism of NK cell specific PLCG2 G595R haploinsufficiency as well as other reported PLCG2 mutations.

6

Antibiotic Altered Microbiota from the Mother Accelerates Development of Colitis in IL-10 Deficient Mice.

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Antibiotic use affects the gut microbiota in humans and animal models, and antibiotic-altered microbiota has been associated with the development of diseases including obesity and atherosclerosis.

Epidemiological studies have associated prior antibiotic use with development of inflammatory bowel diseases (IBD), suggesting that an antibiotic-altered microbiota (AAM) may contribute to IBD development. Based on these findings, we asked whether microbiota from antibiotic-exposed mice could exacerbate colitis in genetically predisposed IL-10 deficient (IL-10-/-) recipients. To address this hypothesis, we obtained already characterized cecal microbial populations: AAM that had been isolated from mice treated with low dose antibiotics, previously shown to alter both metabolic and immunologic phenotypes in germ-free recipient mice, and control microbiota (CM) from untreated mice[1]. These AAM or CM pools were gavaged into pregnant germ-free dams with C57BL/6 wild type (WT) and IL-10 deficient (IL-10-/-) backgrounds. For 21 weeks after birth, fecal samples from their pups were collected and IgA and calprotectin were measured at multiple time points. In addition, fecal 16S rRNA sequencing was performed to assess the transfer efficacy and bacterial population structure in the recipients. Our results show successful transfer of bacteria populations from the pregnant dams to their pups, with congruence in population structures in relation to inoculum (AAM or CM) and to host genotype (WT or IL10-/-). As expected, fecal calprotectin levels were significantly higher in IL-10 -/- compared to WT mice. In WT mice, calprotectin was significantly higher in recipients of AAM compared to CM at week 7. At week 19, IgA concentration was significantly elevated in IL-10 -/- mice compared to WT. However IL-10-/- mice that received AAM had significantly lower IgA levels than those that received CM. Histological staining of colon tissues at sacrifice (week 21) was consistent with colitis development in IL-10 -/-. Notably, the IL-10 -/- mice that received AAM had significantly higher inflammation, hyperplasia, and dysplasia scores than those that received CM. Examination of microbiota in the IL10 -/- mice prior to the histological findings identified specific taxa associated with either acceleration of/or protection from colitis. In summary, our results provide evidence that maternal transfer of an antibiotic-altered microbiota has increased pathogenic effects in offspring predisposed to colitis.

7

Lymphatic Filariasis: Host and Parasite Factors and the Pathogenesis of Systemic Adverse Events Following Treatment

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Lymphatic filariasis (LF, aka “elephantiasis”) is a neglected tropical disease (NTD) that is caused by the nematode parasite *Wuchereria bancrofti.* Some 800 million people in 73 countries are at risk for infection and disability caused by these parasites. Mild to moderate systemic adverse events (AEs) such as fever, myalgia, and headache are common after treatment of LF, and these AEs pose a major challenge for the global LF elimination program that is using mass drug administration (MDA) to interrupt transmission of the disease. We are studying the pathogenesis of AEs with blood samples collected before and after treatment in infected volunteers in clinical trials in Côte d’Ivoire (CdI) and Papua New Guinea (PNG). We have used a Bio-Plex cytokine panel to measure 27 cytokines in 24 LF-infected individuals from PNG at seven time-points, from pre-treatment up until 72 hours post-treatment. Results show that 19 out of the 27 cytokines were significantly increased in post-treatment plasma in individuals with moderate AEs compared to individuals with no/or mild AEs. This included three main pro-inflammatory cytokines (IL-6, TNF-α and IL-1β) that were all increased in people with moderate AEs between 8-36 hours post-treatment. Another interesting, and unexpected result was observed for Eotaxin-1. This eosinophil-specific chemokine was significantly up-regulated at baseline in individuals that would go on to develop moderate AEs after treatment. Eotaxin-1 could therefore be a potential biomarker for AEs. We were able to confirm some of these results in the CdI cohort, including the increased Eotaxin-1 at baseline in people who would develop AEs post-treatment. Preliminary results from global gene expression studies (RNAseq) of 24 individuals from the CdI cohort suggest that several immune pathways are up-regulated in host leukocytes following treatment, and we hope to identify specific transcriptional signatures that are associated with AEs. In addition to studying the host immune response during AEs, we have also measured various parasite components in pre and post-treatment plasma in people with and without AEs. We have found that filarial antigen levels significantly increase at 24 hours post-treatment, and this increase is greater in individuals with AEs. Additionally we have found that filarial DNA can be detected in plasma within 8 hours of treatment, whereas filarial DNA is mostly undetected pre-treatment. These results indicate that filarial parasites are disintegrating after anti-filarial treatment, and we hypothesize that these released parasite components contribute to the development of AEs by activating the immune system, and producing a pro-inflammatory response. Improved understanding of the causes of post-treatment AEs may lead to improved methods for their prevention or management and increase compliance in mass drug administration programs that aim to eliminate LF.

8

Modeling Tuberous Sclerosis Complex using Patient-derived Cells

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Tuberous Sclerosis Complex (TSC) is a pediatric autosomal dominant genetic disorder which causes benign tumor growths in multiple organ systems including the brain, kidney, heart, skin, and lungs. The most debilitating symptoms are a consequence of the brain involvement leading to a high rate of epilepsy, autism spectrum disorder, and learning disabilities. Patients carry a heterozygous mutation in either the *TSC1* or *TSC2* genes, which code for the proteins hamartin and tuberin, respectively. These proteins act upstream of mTOR (mammalian target of rapamycin) complex 1 and complex 2 to regulate multiple processes including cell growth and protein translation. Developmental brain abnormalities have been detected in TSC patients as early as twenty weeks gestation suggesting an important developmental role for hamartin and tuberin. The benign hamartomatous growths in the kidneys and lungs are thought to arise from a somatic mutation in the second allele of *TSC1* or *TSC2*, leading to increased mTORC1 activity and unhindered growth, but leaving the surrounding heterozygous tissue functionally normal. By contrast, emerging data from surgically resected neural hamartomas demonstrate very few cells that show loss of heterozygosity. A major question remains: do heterozygous mutant cells contribute to the pathology of TSC? Patient-derived cell lines present a unique opportunity to explore this question. In the process of generating *TSC2* mutant and wild-type induced pluripotent stem (iPS) lines from human fibroblasts we observed an increased

rate of integration of the reprogramming plasmids in mutant lines. Integrated cell lines retain the shP53/*OCT4* plasmid at a higher rate than the *KLF4/SOX2* or *L-MYC* reprogramming plasmids, suggesting that either the *OCT4* gene or shP53 is driving integration of this plasmid specifically. *We hypothesize that heterozygous loss of TSC2 in human cells is sufficient to alter p53 activity thereby impairing fibroblast reprogramming and driving integration of the shp53 reprogramming plasmid. TSC2+/-* fibroblasts form fewer pluripotent colonies following plasmid reprogramming. Further, *TSC2+/-* fibroblasts display increased p53 protein levels in response to DNA damage. These data support a model where heterozygous loss of *TSC2* in human cells is sufficient to alter cell signaling and function.

9

Development of Muscularis Macrophages in Mice Lacking an Enteric Nervous System

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Interactions between tissue resident muscularis macrophages (MM) and the nervous system of the bowel influence bowel motility and MM inflammatory response. In adult mouse bowel, enteric neurons also appear to be the main local source of colony stimulating factor 1 (CSF1), a protein required for MM survival. Surprisingly, we find that during normal development, MM are observed in the bowel prior to the appearance of enteric neurons. This calls into question the requirement of neuron-derived CSF1 for MM colonization of the bowel. To determine if intestinal innervation is required for MM development, we analyzed MM of neonatal *Ret* knockout (*Ret* KO) mice that have no enteric nervous system (ENS) in the small bowel or colon and have defects in sympathetic innervation. We found normal numbers of well-patterned MM in *Ret* KO bowel. Similarly, the abundance and distribution of MM in aganglionic human colon tissue obtained from Hirschsprung's disease patients was also normal. By profiling gene expression in the ENS and surrounding bowel, we demonstrate that enteric neurons are not the main source of CSF1 in the developing bowel. Additionally, we show that MM from neonatal *Ret* KOs do not differ from controls in their baseline activation status or in their cytokine-production response to lipopolysaccharide (LPS). These data demonstrate that the ENS is dispensable for MM colonization and patterning of the bowel and suggest that any modulatory interactions between MM and the bowel nervous system are established postnatally.

10

Inhibition of the Mitochondrial Pyruvate Carrier Suppresses Oxidative Phosphorylation and Proliferation in Castrate-Resistant Prostate Cancer

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Specific metabolic underpinnings of androgen receptor (AR)-driven growth in prostate cancer are largely undefined, hindering the development of strategies to leverage the metabolic dependencies of this disease when hormonal manipulations fail. To this end, we discovered a subunit of the mitochondrial pyruvate carrier (MPC), *MPC2*, is a direct AR target gene that is increased in hormonally responsive and castrate-resistant prostate cancer and is associated with poor clinical outcomes. Experimental MPC inhibition delays proliferation and alters metabolic outputs of the citric acid cycle (TCA) in AR-driven PCa models. Metabolic disruption owing to MPC inhibition results in activation of the eIF2α/ATF4 integrated stress response to partially compensate for MPC inhibition by coordinating increased glutamine incorporation into the TCA. Glutamine restriction during MPC inhibition resulted in profound TCA disruption and proliferative arrest. Collectively, our findings characterize the MPC as a facultative component of prostate tumor metabolism and support further examination of the MPC as a potential therapeutic target in additional tumor types.

11

ABCB5 Promotes Cutaneous Wound Healing through Regulation of a Pro-angiogenic pAKT/HIF1A/VEGF Signaling Cascade

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ABCB5 (ATP-binding cassette, sub-family B (MDR/TAP), member 5) has recently been shown to identify limbal stem cell populations in the mammalian eye and to regulate corneal epithelial development and repair. ABCB5 is also expressed by subpopulations of dermal mesenchymal stem cells (MSC) in human and murine skin, but its potential role in cutaneous repair is currently unknown. Here we show that ABCB5 is functionally required for normal cutaneous regeneration, and that ABCB5(+) MSC possess therapeutic capacity to enhance regenerative wound healing responses through induction of pro-angiogenic molecular pathways. Histological analyses of a series of human clinical specimens revealed a significant decline of dermal ABCB5(+) MSC frequencies in the skin of older-aged (>70 years) healthy individuals and enhanced ABCB5(+) MSC frequencies during scaffold-induced regenerative wound healing, suggesting an essential role of ABCB5 in more efficient cutaneous repair. In support of this hypothesis, xenotransplantation of human ABCB5(+) MSC grafts to immunocompromised NSG mouse recipients in a full thickness skin wounding model resulted in accelerated wound closure, decreased inflammatory stroma thickness and enhanced microvessel density with induction of pro-angiogenic pAKT, HIF1A, and VEGF signaling and augmented expression of the endothelial marker, CD31. The functional role of ABCB5 in cutaneous wound healing was further mechanistically dissected in a novel Abcb5 knockout (KO) mouse model of cutaneous wound repair, which revealed, as a corollary to human xenotransplantation findings, impaired pAKT/HIF1A/VEGF signaling associated with delayed wound closure, increased inflammatory stroma thickness, and decreased Cd31-positive vessel formation in Abcb5 KO versus wildtype mice. Our results demonstrate that ABCB5 promotes mammalian cutaneous wound healing through regulation of a pro-angiogenic pAKT/HIF1A/VEGF signaling cascade and suggest in a translationally relevant xenotransplantation model therapeutic potential of human ABCB5(+) MSC for augmentation of wound repair.

12

Thiazolidinediones Suppress the Allergic Th2 Response by Controlling Mitochondrial Metabolism

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Allergy is a class of often debilitating diseases that reflects maladaptive Th2 immune responses. In an effort to identify novel druggable targets to specifically abrogate Th2 immunity in the context of allergy, we conducted transcriptomic analyses of Th1, Th2, and Th17 cells and found PPARg to be highly expressed in Th2 cells. Upon treatment with the PPARg agonists thiazolidinediones (TZDs), Th2 effector function, as measured by IL-4 and IL-13 cytokine expression, is decreased *in vitro*, and mice treated with TZDs are protected from skin allergy *in vivo*. We find that TZDs induce mitochondrial biogenesis in Th2 cells, which undergirds a coordinated nutrient switch from glucose to fatty acids. The induced increase in fatty acid catabolism is essential for the suppression of the Th2 immune response. Taken together, our data illustrates the importance of nutrient selectivity and metabolism in T cell effector functions and argues for the use of TZDs in treatment of Th2 driven disease by modulation of nutrient selectivity and metabolism, principles that can be leveraged to protect against other Th driven diseases.

13

A Genomic Screen identifies Genes Essential for the Anaerobic Survival of the Opportunistic Pathogen *Pseudomonas Aeruginosa*

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Microbial growth arrest is a ubiquitous yet understudied phenomenon. Of particular concern to an aerobic microbe like *Pseudomonas aeruginosa* is the depletion of oxygen as a respiratory terminal electron acceptor. The relevance of this growth-arresting condition is becoming more appreciated in the context of biofilms, cystic fibrosis (CF) lung disease, and chronic wounds. For instance, oxygen concentrations are routinely in the nanomolar range in CF sputum, and genes for anaerobic metabolism are upregulated in *P. aeruginosa* in vivo, suggesting that this organism must regularly adapt to an anaerobic lung environment. Despite their relevance to chronic infection, the molecular responses of *P. aeruginosa* to hypoxia-induced growth arrest are largely unknown. Characterizing these responses is important not only for a better understanding of basic microbial physiology, but also for a better understanding of the factors that contribute to chronic infections caused by this organism. To begin investigating the molecular mechanisms at play during hypoxia-induced growth arrest, we performed a genome-wide fitness screen using transposon insertion sequencing (Tn-seq) to identify genes that contribute to fitness of *P. aeruginosa* during anaerobic survival on pyruvate. Tn-seq uses the power of massively parallel sequencing to quantify changes in relative abundance of insertion mutants in a user-generated transposon mutant pool under a condition of interest. These changes in abundance are used to determine mutant fitness and thus the contribution of the disrupted gene to growth and survival. Our screen identified the sigma factor RpoS and putative members of its regulon as essential for anaerobic survival, revealing a novel functional role for RpoS in *P. aeruginosa* physiology. Overall, this experiment helps validate Tn-seq as a high throughput, unbiased, quantitative approach for interrogating the genome-wide fitness of organisms under growth-arrested states.

14

Cervical Vagus Nerve Senses Changes in Blood Glucose Homeostasis

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The central nervous system plays a critical role in maintaining energy and glucose homeostasis. The vagus nerve senses changes in systemic glucose levels and transmits the information to the central nervous system. The efferent signals generated in response are then transmitted back to the periphery to regulate glucose homeostasis. In the current study, our goal is to leverage the information transmitted in the vagus nerve, record and decode the neural signals generated in response to metabolic changes, and use the information to develop devices that can monitor and potentially control blood glucose levels in diabetic patients. We have recorded neural activity from the cervical vagus nerve of 19 mice while simultaneously manipulating and tracking acute changes in blood glucose levels. Using novel signal processing and feature extraction methods, we can isolate and track discrete neural features over time. Lagged, multivariate linear regression-based methods accurately correlate and predict blood glucose levels using only these neural features. These correlations indicate that information in vagus nerve activity can be used to monitor changes in blood glucose levels. Decoding these signals with machine learning algorithms will enable us to predict these changes in real time. Moreover, functional and anatomical mapping of this reciprocal signaling will identify the properties of the neural circuit underlying the regulation of blood glucose levels, and provide a therapeutic target for the treatment of diabetes.

15

Characteristics of Acute Compartment Syndrome in the Forearm after Gunshot Trauma

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Acute compartment syndrome of the upper extremity is a surgical emergency which requires prompt intervention. Delay in diagnosis or intervention can result in devastating consequences including neurologic dysfunction, ischemic contractures, muscle dysfunction and loss of life or limb. Penetrating injuries causing compartment syndrome have been largely ignored in the current literature, yet are a significant burden within many urban centers. The objective of this study was to determine causes, complications, and clinical outcomes associated with forearm compartment syndrome of the upper extremity resulting from gunshot wounds. **Results:** Twenty-five patients who underwent forearm fasciotomies due to gunshot were identified over a 14-year period. All patients were males with a mean age of 28 years old. Seventy-two percent (n=18) suffered the gunshot wound to the elbow region, and 60% (n=15) suffered an associated fracture. Injury to a named artery occurred in 48%, with the most common injury involving the brachial artery. A clinical diagnosis (with or without Stryker monitor) was made in 14 cases,

and made with Stryker monitor alone in 7 cases (5 of these patients were intubated, and 2 patients had an unreliable exam due to nerve injury). Neurologic sensory and motor deficits were noted in 13 and 6 patients respectively prior to fasciotomy, with 38% (n=8) recovering full sensory function and 33% (n =2) recovering full motor function after fasciotomy. A mean of 2.7 operative procedures were performed for wound management (I&D, closure, etc.) (range 0-23, SD-4.4) after fasciotomy, with 12 patients (48%) requiring skin graft or free flap coverage. Only 20% of patients recovered full painless function on most recent follow up, with the remaining 80% suffering from one or more of the following: neurologic deficit (56%), Chronic pain in extremity (28%), decreased range of motion (32%). Discussion Forearm compartment syndrome from a gunshot is a difficult diagnosis due to its association with fractures, soft tissue damage, and neurovascular injury. Diligent and frequent clinical examination should be performed in all individuals with abnormal vascular status. Pressure monitoring should be considered in all intubated patients, and those who have altered sensation. Acute compartment syndrome of the forearm has significant morbidity often with permanent neurologic injury and loss of function. Emergent fasciotomy is required to decompress the forearm musculature and maximize patient outcomes.

16

Keeping Patient Care Alive While Performing PhD Research: MSTP Students and Clinical Skills.

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School of Medicine students, pursuing both MD and PhD degrees in Medical Scientist Training (MST) Program, attend medical school for two years before spending 3-5 years performing research. Upon receiving their PhD, MSTP students return to the Medical School for clerkship rotations. The transition back to medical training is often stressful due to the prolonged separation from clinical education. A survey of UVA MSTP students showed that >70% said they would benefit from more integration of medical and graduate education and 95% of respondents said they would be interested in attending clinical skills retention sessions during their graduate training. Thus, MSTP students at UVA have collaborated with the Clinical Skills Center to develop a bimonthly clinical skills review curriculum for MSTP students in their graduate years to mitigate the inherent stress of transitioning back to medical school and increase retention of clinical knowledge. Five sessions throughout the year cover the main aspects of the physical examination and the sixth session is a full-length Objective Standardized Clinical Examination (OSCE). Each review session focuses on an aspect of the physical exam (e.g., Pulmonary exam). The format for the review sessions is as follows: 1) A 30-minute orientation session explaining the important information for obtaining a detailed patient history and review of systems, the pertinent physical exam techniques, and key information to include in a presentation. 2) The students then move in small groups between mock patient rooms where they will actively participate in a clinical encounter, which will involve unique presentations requiring the physical examination reviewed during orientation (e.g., for pulmonary exam: pneumonia, lung cancer, and GERD). Each case will involve taking a detailed history, completing a pertinent physical exam, and presenting the patient. A 3rd or 4th-year medical student will supervise each case, acting as the patient until the end of the case where they assume the role of the physician. By the end of the year we have covered all the physical examination techniques expected of a third-year medical student entering clerkships. We have recently concluded the first full year of clinical review sessions and the response has been overwhelmingly positive. It is our hope that by providing consistent, active clinical encounters during the graduate years of the UVA MD/PhD curriculum, we will improve the integration of the medical and graduate education to better prepare students for the transition back to medical school as meaningful members of the patient care team.

17

Proximal Collimation to Apply Non-synchrotron Microbeam Radiotherapy

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The treatment of brain cancers, either primary or metastatic tumors, remains a therapeutic dilemma with significant morbidity and mortality. Tumor-blood-brain barrier reduces the efficacy of conventional chemotherapies, so radiation therapy remains the major treatment for these patients. Normal tissue toxicity is the primary limiting side effect of radiotherapy, though. Microbeam radiotherapy (MRT) is a new preclinical modality that delivers spatially fractionated submillimeter quasi-parallel ultra high-dose of orthovoltage X-ray beams (peak), separated by wider non-irradiated regions (valley). Studies consistently demonstrate higher tumoricidal effects of MRT than conventional broad-beam radiotherapy (BB), while sparing highly sensitive normal tissue, namely normal brain tissue. High intense monochromatic parallel X-ray photons generated by synchrotron sources have the ideal characteristics for applying less than 100μm microbeams and, as a result, most of the MRT studies have been confined to beams thinner than 100μm and done in sparsely distributed synchrotron facilities. However, given the sparsity and limited access to these systems, we have sought to develop a non-synchrotron based approach capable of producing similar microbeam dosimetry in order to facilitate the translation of this technology. We have developed a collimator that is able to convert the cone beam of an industrial irradiator (160 kVp, 2mm Al filter) to 44 identical 300μm beam, with the center-to-center distance of 900μm (irradiation field=39mm). The dose rate is 2.9 Gy/min at the source-to-surface distance of 37cm. The peak-to-valley dose ratio is more than 24 at entrance plan (comparable to synchrotron). The response of two tumor cell lines [B16-F10(melanoma), TRP(glioblastoma)] to MRT (20 -172 Gy) and BB (2 –15 Gy) irradiation was investigated using gold standard clonogenic assay. MRT was performed using our in-house made collimator. BB irradiations were performed using the same irradiator after removing the collimator. Dose equivalence between MRT and BB were verified in vitro in two cell lines. We found for example, that BB dose of 7.6 Gy on B16-F10 cells was radiobiological equivalent to a peak microbeam dose of 57.1±0.7 Gy. Our data provides the first determination of biological dose equivalence between BB and non-synchrotron MRT modalities for different cell lines using clonogenic assay. These results will be useful for the dose selection of MRT for future in vivo studies.

18

Metabolic Barriers to T Cell Function and Immunotherapy in Renal Cell Carcinoma

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Cancer cells can inhibit effector T cells through both immunomodulatory receptors and alteration of the tumor microenvironment as a result of cancer metabolism. Renal cell carcinoma (RCC) has long been acknowledged as an immunologically-sensitive tumor for which immune modulator therapies have shown promise. However, a majority of patients treated with immune checkpoint inhibitors recently approved by the FDA fail to exhibit a clinical response. The extent to which metabolic conditions within the tumor impede T cell activation and anti-tumor effector function in RCC are unknown. Through work with Rag deficient mice lacking functional B and T cells, we have established that tumor growth is regulated in a T cell dependent manner as evidenced by earlier formation and faster tumor growth. In a syngeneic mouse model of renal cell carcinoma (RenCa), we find that inhibition of PD-1, relieving negative regulation of T cells, delays tumor growth and size. We have also analyzed human patient-derived renal cell carcinoma tumors and found that metabolism of tumor infiltrating lymphocytes (TILs) and, specifically, CD8 effector cells is significantly altered. CD8 TILs were abundant in RCC, but are phenotypically distinct and are impaired both functionally and metabolically. Instead of efficient use of aerobic glycolysis, TILs fail to increase glucose metabolism, and instead display increased reactive oxygen species (ROS) and mitochondrial dysfunction. CD8 effector cells found in tumors have notable differences in mitochondrial morphology compared to healthy control CD8 T cells by electron microscopy, correlating to findings of hyper-polarized membranes and increased ROS. Bypassing glycolytic defects using pyruvate or neutralizing mitochondrial ROS with scavengers can partially restore CD8 activation. Approximately 25% of patients with RCC respond to immune modulatory therapy with checkpoint inhibitors and we hope that, with improved understanding of the interaction between RCC and the immune system, we can develop combined therapies to improve this response rate.

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Search for Fibrogenic Mesenchymal Progenitor Cells in Idiopathic Pulmonary Fibrosis Through Single-Cell RNA-Seq

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Idiopathic Pulmonary Fibrosis (IPF) is a fatal disease in which activated myofibroblasts mediate progressive fibrosis of the gas-exchange apparatus. Although the biology of the bulk IPF myofibroblast population has been extensively studied, questions remain about the origin and maintenance of this relentlessly profibrotic cell population. We have identified mesenchymal progenitor cells (MPCs) from IPF patient lung explants (identified by the cell surface marker stage-specific embryonic antigen 4 (SSEA4)) whose progeny display the IPF myofibroblast profibrotic signaling signature, and form fibrotic lesions in both zebrafish and mouse xenograft models. To gain further insights into the biology of IPF MPCs, we initiated a series of experiments to isolate and characterize fibrogenic IPF MPCs at the molecular level. Based on prior knowledge that the fibrosis in IPF is heterogeneous in time and space we began with the hypothesis that the IPF lung contains at least 3 subpopulations of MPCs: 1) normal MPCs residing in what appear to be uninvolved alveoli; 2) classically activated MPCs in areas displaying epithelial injury (expected to be identical to MPCs found after tissue injury, whose progeny are dependent on external cues from immune cells and ECM to mediate fibrosis); and 3) fibrogenic MPCs (whose progeny mediate fibrosis in a cell-autonomous manner). To detect MPC heterogeneity, we performed single-cell whole transcriptome RNA-seq on lung explant SSEA4+ MPCs isolated from IPF patients (n = 3) and patient controls (n = 3). Hierarchical clustering analysis revealed putative cell surface proteins that correlate with MPC clusters displaying profibrotic transcriptional profiles. Global analysis of gene-gene interactions revealed a highly-connected network of differentially expressed genes in IPF compared to control MPCs. This network contained genes mapping to fibrosis-relevant ontologies such as ECM production and sensing. Immunolocalization studies are in progress to identify the precise position of cells in different subpopulations. Based on our preliminary analysis, it is clear that the SSEA4+, IPF MPC population exhibits significant heterogeneity, with sub-populations exhibiting a pro-fibrotic transcriptome. Further bioinformatic analysis will seek to identify post-transcriptional mechanisms that mediate the gene expression changes observed among IPF MPC subpopulations. Our overarching goal is to definitively identify, localize, isolate, and characterize the pathogenic cell-of-origin of activated myofibroblasts in IPF, with the ultimate goal of identifying molecular targets for drug discovery.

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Loss of the Mitochondrial Pyruvate Carrier Increases Susceptibility to Tumorigenesis in the Mouse Colon

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The lifetime risk for developing colorectal cancer is approximately 1 in 27 for the American patient. Certain metabolic adaptations, such as loss of the mitochondrial pyruvate carrier (MPC), pre-dispose a cell towards proliferation and can have prognostic value in colorectal cancer. Through a genetic knockout of MPC in Lrig1+ colon stem cells, we have observed that loss of MPC increases proliferation zones in the colon crypts *in vivo.* Loss of MPC expands the stem cell compartment *in vivo* as seen through increased numbers of Ki67+ and BrDU+ cells in histology. Markers of stemness in the murine colon and in intestinal organoids also increase, likely through cell autonomous changes mediated by metabolite flux. We have strong evidence that loss of MPC alone in colon stem cells is insufficient to initiate adenomatous transformation in this mouse model. Thus, we are investigating if loss of MPC delays differentiation to achieve the hyperproliferative phenotype. To explore whether loss of MPC contributes to tumorigenesis by pre-establishing the Warburg effect, we implemented the azoxymethane/dextran-sodium-sulfate (AOM-DSS) chemical method of colon cancer induction. AOM-DSS challenges the colon selectively with mutagenesis and colitis in a reproducible manner. Chemical induction of colorectal cancer in MPC knockout colons increases polyp formation and polyp histological grade compared to wildtype controls, with no effect on the whole body metabolism or composition, nor response to AOM-DSS treatment itself. Further studies are underway to elucidate if MPC knockout polyps have a unique molecular signature than MPC

wildtype polyps. Preliminarily, total β-catenin levels appear lower in MPC knockout polyps compared to wildtype polyps, suggesting that gain of function Wnt signaling may not be as necessary in MPC knockouts. These investigations into the coupling of glucose metabolism with mitochondrial oxidation will guide a greater understanding of how metabolism influences cell state, particularly in proliferation and renewal of stem cells vs. cancer cells.

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Using Calcium-activated Chloride Channel Regulator 1 (CLCA1) to Activate Alternate Anion Currents in Cystic Fibrosis Airway

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Cystic fibrosis (CF) is a genetic disorder primarily affecting the lung that results from mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. Roughly 2000 potentially causative mutations have been currently identified in *CFTR* thought to result in impaired chloride secretion. There has been success in the development of therapies for two specific mutations, deltaF508 and G551D. However, developing targeted therapies for the thousands of identified mutations would be challenging. Another strategy may involve activating an alternate chloride channel in lung epithelium to bypass CFTR dysfunction. Using whole cell patch clamp and confocal microscopy, our group has recently clarified a mechanism by which CLCA1 (calcium-activated chloride channel regulator 1) specifically activates chloride currents through the calcium activated chloride channel in the lung, TMEM16A: by increasing TMEM16A surface localization and reducing its trafficking on the order of minutes. Additional experiments show that CLCA1 activation of these currents can be localized to its von Willebrand type A (vWA) domain, though it may be metal ion-dependent adhesion site (MIDAS) independent. Given the specificity of CLCA1 in the activation of TMEM16A, we aimed to utilize CLCA1 to activate alternate anion currents in CF airway epithelium. This strategy has the potential to restore chloride currents in CF patients regardless of genotype. To examine the localization of TMEM16A in the lung, we stained CF lung tissue sections (genotype homozygous deltaF508) with anti-TMEM16A antibody. As expected, low levels of expression were found in apical airway epithelium as well as in submucosal glands. To begin to test whether CLCA1 may activate TMEM16A currents in CF, we obtained CF airway epithelium (genotype delF508/2789+5G>A), applied vector or CLCA1-conditioned media to these cells for 24 hours and subsequently performed whole-cell patch clamp. CLCA1-conditioned media was found to significantly increase chloride currents above control. Furthermore, when TMEM16A inhibitor $T16A_{inh}$ was applied to cells receiving CLCA1-conditioned media, current was reduced to control levels. These preliminary results indicate that CLCA1 can specifically increase chloride currents in CF airway epithelium with a heterozygous genotype. Further studies will be required to investigate whether activation of TMEM16A currents using CLCA1 can increase chloride currents in CF airway epithelium of different genotypes and whether it may restore healthy mucous properties.

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BDNF in Peripheral Fluids as a Biomarker for Alzheimer's Disease

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Brain-derived neurotrophic factor (BDNF) is a growth factor that is critical for the development, maintenance, and survival of new neurons and synapses. Previous studies have linked dysregulation of BDNF to depression and schizophrenia, and a growing body of literature has implicated the disruption of BDNF biosynthesis in the pathogenesis of Alzheimer's disease (AD). In particular, BDNF has been shown to have a dose-dependent protective effect against amyloid toxicity in AD mouse models. Additionally, reduction in levels of BDNF has been correlated with the accumulation of neuritic amyloid plaques in humans with AD. BDNF effects on AD pathology make this protein a promising potential biomarker for this disease and, as a result, BDNF levels in blood have been explored to detect and monitor the course of pathological aging. However, the relationship between peripheral measures of BDNF (e.g., from serum or plasma) and levels of BDNF in the brain have not been thoroughly investigated in human samples. Previous reports relating BDNF levels in serum to neuropathological conditions have been conflicting, and some studies report higher BDNF levels while others report lower BDNF levels in AD. These discrepancies may exist because BDNF in peripheral fluids have non-neuronal origins which suggest that better methods to isolate and measure BDNF are needed. In this study, we use post-mortem brain and fluid samples from AD patients and age-matched non-impaired donors to investigate whether different sources of BDNF measurements reflect brain levels of BDNF. We also used a novel technique to isolate nanometer-sized vesicles from blood that are secreted by neurons called neuron-derived exosomes. Our preliminary results suggest that levels of BDNF in serum do not correlate with BDNF levels in the brain of control and AD patients. However, we found that AD pathology (i.e., levels of Aβ42 and pTau) in neuron-derived exosomes was highly correlated with BDNF levels in brain tissue. This work is supported by a grant from the National Institute on Aging (R21-AG048631).

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Elevated Glutaminase and Aspartate Aminotransferase in Rat DRG Neurons During Adjuvant-induced Arthritis

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The sensory neurons of the dorsal root ganglia (DRG) are glutamatergic, utilizing glutaminase (GLS) and aspartate aminotransferase (AST) for glutamate synthesis (Miller et al., Pharmacol Ther 130:283, 2011). We previously demonstrated that glutamate metabolism (↑ GLS, AST, glutamate levels) is altered in the rat DRG, sciatic nerve and skin during adjuvant-induced arthritis (AIA). Additionally, we have determined that AST is elevated in nociceptive, calcitonin gene-related peptide- (CGRP) containing DRG neurons during AIA. The aim of the present study is to evaluate alterations of GLS in AST-containing DRG neurons during AIA. Complete Freund's adjuvant was injected into the rat right hindpaw to induce AIA and DRG's were analyzed at 2, 4 and 8 days. Rats were

anesthetized and transcardially perfused with fixative. AST and GLS colocalization was evaluated in L4 DRG's with immunohistochemistry, followed by quantitative image analysis with Image J. At 2 and 8 days AIA, GLS-immunoreactivity (ir) in AST-positive DRG neurons was elevated when compared to naïve controls, demonstrating a biphasic pattern of expression. In a similar biphasic pattern, AST-ir in GLS-positive neurons was elevated at days 2 and 8 of AIA, but was a control levels at day 4 AIA. Analysis demonstrated strong positive correlation in GLS-AST elevation in DRG neurons at 2 and 8 days of AIA. Elevated AST and GLS levels in DRG neuronal bodies leads to increased glutamate production in peripheral and central terminals. The elevation observed for both AST and GLS, therefore, may be due to similar mechanisms regulating transcription, translation, and trafficking in DRG neurons. Novel therapies that decrease AST and GLS levels may hold promising treatment for alleviating inflammatory pain caused by increased glutamate synthesis in nociceptive primary afferents following injury.

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Th17 Cells are Refractory to Senescence Retaining Robust Antitumor Activity afterLong-term *Ex Vivo* Expansion

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Current dogma suggests that adoptive T cell therapy for advanced solid tumors relies on infusing large numbers of T cells to mediate successful antitumor immunity. Yet, $CD8^+$ T cells experience a decline in fitness and antitumor efficacy during the rapid *in vitro* expansion protocols that generate sufficient numbers for treatment. In contrast, we discovered that IL-17-producing $CD4^+$ T cells (Th17 cells) expand 5,000-fold during *in vitro* expansion and unlike Th1 cells, do not gain or exhibit hallmarks of senescence or apoptosis but instead have preserved antitumor efficacy *in vivo.* Three-week expanded Th17 cells engrafted into host mice and eliminated melanoma as effectively as Th17 cells expanded for one week when infused in equal numbers. However, treating large, recalcitrant tumors, required the infusion of all cells generated after two or three weeks of expansion, while the cell-yield obtained after one week of expansion was insufficient. Additionally, long-term expanded Th17 cell products bestowed protection from tumor re-challenge and produced sustained cures. Importantly, long-term expanded Th17-polarized human $CD4^+$ T cells also exhibit durable antitumor efficacy compared to Th1-polarized cells suggesting that Th17 cells offer a rare ability to ablate highly aggressive tumors due to resistance to senescence.

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$CD301b^+$ $SIRP\alpha^+$ Dendritic Cells are Localized Exclusively Within the Thymus Medulla

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Negative selection removes T cells with high affinity self-interactions that may result in self-reactivity upon thymic emigration. The thymic medulla is considered a specialized site for negative selection because of AIRE (autoimmune regulator)-mediated expression of tissue-specific antigens, and because dendritic cells (DC) are enriched in the medulla. Dendritic cells represent an extremely heterogeneous population of antigen presenting cells within the thymus. $SIRP\alpha^+$ DC recirculate to the thymus and display self-antigens acquired in the periphery, whereas $XCR1^+$ DC cross-present tissue-specific antigens acquired from medullary thymic epithelial cells. These unique functional capacities suggest that thymic DC play non-redundant roles in facilitating central tolerance. Analysis of DC populations within the thymus revealed that a substantial proportion of $SIRP\alpha^+$ DC within the thymus are $CD301b^+$. Further visualization of this subset with immunofluorescence microscopy demonstrated that $CD301b^+$ $SIRP\alpha^+$ DC are localized exclusively within the thymic medulla, whereas $CD301b^-$ $SIRP\alpha^+$ DC are dispersed throughout the cortex. To selectively deplete $CD301b^+$ $SIRP\alpha^+$ DC in vivo, $Mgl2^{DTR\text{-}GFP}$ mice are being utilized to analyze the role of this DC subset in mediating clonal deletion and Treg generation. These data suggest that $CD301b^+$ $SIRP\alpha^+$ DC represent a unique population of antigen presenting cells within the thymus. Furthermore, the distinct localization of the two migratory $SIRP\alpha^+$ DC populations within the thymus indicates that they play non-overlapping roles in mediating central tolerance. These findings will be important to understanding how autoimmunity to distinct antigens develops.

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Regulation of the Nuclear and Mitochondrial Genome by the Putative Oncometabolite L-2-Hydroxyglutarate

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Clear cell renal cell carcinoma (ccRCC) is the most common form of kidney cancer and is associated with two chromosomal deletions, 3p and 14q. Although loss of 14q is associated with a worsened prognosis, the reasons for this remain unclear. L-2-Hydroxyglutarate Dehydrogenase (L2HGDH) is located on chromosome 14 and is often lost in these patients. L2HGDH deficiency leads to an accumulation of L-2-Hydroxyglutarate (L-2HG) by way of the inability to counter the off-target activity of dehydrogenases including malate dehydrogenase (MDH). Here, we provide insight into the underlying biochemistry of L-2HG in the context of ccRCC and the biologic implications of lowering this metabolite using both *in vitro* and *in vivo* models. In addition, through the analysis of patient samples, cell line models, and a CRISPR knockout mouse, we provide evidence that this small molecule can regulate the expression of both nuclear and mitochondrial DNA encoded genes. Collectively, our data provide novel insight into the epigenetic targets of L-2HG and provide new opportunities for the treatment of L-2HG driven tumors.

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IRF4(+) Dendritic Cells Promote Allergic Th2 Responses in the Lungs

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A role for dendritic cells (DCs) in promoting adaptive immune responses is well-appreciated, but the specific mechanisms by which DCs initiate Th2 responses are not completely understood. We previously found that mice lacking the transcription factor IRF4 in DCs do not develop type 2 lung inflammation in response to house dust mite extract (HDM). Further, T cells restimulated with HDM by IRF4-deficient DCs were deficient in Th2 polarization in ex vivo cultures. We hypothesized that IRF4 is necessary for DCs to perform one or more of the many functions required to successfully initiate T cell responses in vivo. Here we demonstrate that IRF4 in DCs is not required for DC uptake of allergen in the lungs, nor for the presence of HDM-bearing DCs in the lung-draining lymph nodes (LLNs) during HDM sensitization. Similarly, IRF4-deficient DCs are capable of antigen processing. However, IRF4-deficient CD24(+) CD11b(+) cDCs express lower levels of the Th2-associated costimulatory molecule OX40L during in vivo HDM sensitization. CD4(+) effector/memory T cell recruitment to the lungs and CD69 expression by CD4(+) T cells in the lung parenchyma after HDM sensitization is impaired in mice with IRF4-deficient DCs. These findings suggest that DC expression of IRF4 regulates DC-T cell interactions that lead to Th2 differentiation in vivo.

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Computational Model of Antidepressant Response Heterogeneity as Multi-Pathway Neuroadaptation

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Current hypotheses cannot fully explain the clinically observed heterogeneity in antidepressant response. The therapeutic latency of antidepressants suggests that therapeutic outcomes are achieved not by the acute effects of the drugs, but rather by the homeostatic changes that occur as the brain adapts to their chronic administration. We present a computational model that represents the known interactions between the monoaminergic neurotransmitter-producing brain regions and associated non-monoaminergic neurotransmitter systems, and use the model to explore the possible ways in which the brain can homeostatically adjust to chronic antidepressant administration. The model also represents the neuron-specific neurotransmitter receptors that are known to adjust their strengths (expressions or sensitivities) in response to chronic antidepressant administration, and neuroadaptation in the model occurs through sequential adjustments in these receptor strengths. The main result is that the model can reach similar levels of adaptation to chronic administration of the same antidepressant drug or drug combination along many different pathways, arriving correspondingly at many different receptor strength configurations, but not all of those adapted configurations are also associated with therapeutic elevations in monoamine levels. When expressed as the percentage of adapted configurations that are also associated with elevations in one or more of the monoamines, our modeling results largely agree with the percentage efficacy rates of known antidepressants and antidepressant combinations. Our neuroadaptation model provides an explanation for the clinical reports of heterogeneous clinical outcomes among patients chronically administered the same antidepressant drug regimen.

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Statin Induced Cytotoxicity in Embryonic Neural Stem/Progenitor Cells

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Although statins have proven to be safe and effective drugs in combating cardiovascular disease, they are currently contraindicated in pregnancy due to potential teratogenic effects. Statins reduce serum cholesterol by inhibiting the rate-limiting enzyme of cholesterol synthesis, HMG-CoA reductase (HMGR). In the developing neocortex, products of the cholesterol biosynthesis pathway (CBP), including cholesterol and isoprenoids, play essential roles in proliferation and apoptosis of neural stem-progenitor cells (NSPCs). To determine whether statins impact NSPC viability, embryonic day 14.5 primary neurosphere cultures were treated with pravastatin or simvastatin *in vitro* and cell number was monitored over the course of 5 days. We found that both statins dose dependently decrease cell number and neurosphere size. The proliferation of NSPCs treated with low doses of statins (1uM of Pravastatin or 0.1uM Simvastatin) was unaffected, but higher doses of statins (25uM Pravastatin or 5uM Simvastatin) caused a nearly complete inhibition of growth. In order to understand whether this phenotype was due to decreased proliferation or increased cell death, we analyzed cell cycle progression and apoptosis of NSPCs by flow cytometry. We found that statins cause a dose dependent increase in apoptosis, which was validated using Western blot analysis by increased cleavage of the apoptosis marker PARP protein. To understand how NSPCs respond to statins at a transcriptional level, we treated NSPCs with pravastatin and examined global mRNA expression using RNA sequencing technology. Ingenuity Pathway Analysis (IPA) revealed upregulation of CBP genes in addition to identifying dysregulated CBP as the top toxicity function. Statin induction of a number of CBP genes was validated by qRT-PCR. IPA identified SCAP, INSIG, and SREBP2, which form a complex known to regulate CBP genes in peripheral tissues as "top regulators" after statin treatment. Activation of SREBP2, a CBP regulating transcription factor, was detected by western blot, which corroborated the IPA prediction and suggested a likely mechanism for CBP gene induction by statins. Because NSPCs survive low dose statin treatment, we set out to determine whether statin induced transcription of CBP genes was an adaptive response to inhibition of *de novo* cholesterol biosynthesis by statins. We found that a 24hr pretreatment with low dose pravastatin was not sufficient to rescue cell growth in NSPCs treated with statins compared to controls, which suggests that statin induced gene transcription is not protective against chronic statin treatment. Interestingly, an acute 24hr statin treatment did not affect the long term proliferation of NSPCs.

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Genetic Evaluation of Therapeutic Targets in the Insulin Signaling and Adipogenic Pathways

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MAP kinase phosphatase 3 (MKP3), phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase (G6Pase), and histone deacetylases (HDACs) have been implicated in regulating metabolic components of the insulin signaling pathway, adipogenesis, and gluconeogenesis. However, the association between these genes and risk of obesity and type 2 diabetes (T2D) has not been investigated comprehensively before. **Methods:** We examined the roles of MKP3 (DUSP6 in human), PEPCK (PCK2), HDAC1&2, and G6Pase single nucleotide polymorphisms (SNPs) in relation to obesity and diabetes risk among 7,287 African American (AA), 3,258 Hispanic American (HA), and 3711 Caucasian American (CA) women participating in the national Women's Health Initiative (WHI) SNP Health Association Resource (SHARe) and the Genomics and Randomized Trials Network (GARNET). We also replicated the potential findings in the Framingham Heart Study (FHS) and Jackson Heart Study (JHS). **Results:** Our candidate gene study of American women of three ethnicities identified germline mutations in DUSP6, PCK2, HDAC1&2, and G6Pase in relation to obesity and diabetes. **Conclusion:** The human genetic evidence is comparable to data from animal models that support the notion that MKP3, PCK2, HDAC1&2, and G6Pase may serve as important metabolic therapeutic targets in lowering risk of obesity and diabetes.

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The Ubiquitin Proteasome System as a Novel Mechanistic Link between COPD and Atherosclerosis

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Patients with COPD are at greatly increased risk of atherosclerotic vascular disease, however, causal mechanisms linking COPD with atherosclerosis remain elusive. A ubiquitin E3 ligase component FBXO3 has been shown to impact the development of COPD in smokers. Our objective was to determine if FBXO3 also impacts the development of atherosclerosis, and may therefore be a novel mechanism linking COPD with atherosclerosis. **Methods**: Human subjects: Data on 496 smokers enrolled in the SCCOR cohort was analyzed. Major inclusion criteria were age 40-80 years, and >10 pack year smoking history. A hypo functioning genetic variant of FBXO3 was assessed in the 496 participants using extracted genomic DNA from peripheral blood mononuclear cells. Carotid plaque was assessed in 273 participants by ultrasound. In vitro: Formation of foam cells i.e. fat-laden macrophages is critical in atherogenesis. Therefore, we examined FBXO3 in an in vitro model of foam cell formation. We utilized THP-1 cells exposed to 15nM PMA for 48 hours followed by serum starvation for 24 hours. These cells were then incubated with oxidized low-density lipoprotein (oxLDL) that was prepared by exposing native LDL to 40nM CuSO4 for 24 hours. Native LDL was the negative control, while LPS (0.1 ug/mL) was the positive control. **Results:** Human subjects (n=496) were 68.1 ± 6.2 years old and 53.7% male. The hypo functioning genetic variant of FBXO3 was associated with reduced carotid plaque independent of cardiovascular disease risk factors (OR=1.99, p=0.03) as well as lower systolic blood pressure (mean difference 4.7 mm Hg, p= 0.01) independent of demographics and anti-hypertensive medication use. Stimulation of THP-1 cells with 40 ug/mL of oxLDL for 3 hours was associated with an inflammatory response i.e. activation of the NFkB pathway. Secretion of pro-inflammatory mediators was also increased at 24 hours. The time course of activation of the NFkB pathway was different for oxLDL vs. LPS. Phosphorylation of IκBα was maximal at 3 hours with oxLDL while it was maximal at one hour with LPS (0.1 ug/mL). Also, phosphorylation of the p65 subunit occurred at an earlier time point after oxLDL exposure compared with LPS. Stimulation with oxLDL modestly increased levels of FBXO3 protein, and decreased levels of FBXL2 protein (one of the proteins targeted for degradation by FBXO3). Studies to identify the impact of knockdown of FBXO3 using siRNA on the uptake of OxLDL and the associated inflammatory response in these cells are ongoing. **Conclusions**: FBXO3 may contribute to the development of atherosclerosis. Because it has previously been linked with the development of COPD, it may represent a novel mechanistic link between COPD and atherosclerosis.

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The Role of Cdk8/Ssn801 in Mitochondrial Morphology and Virulence of *Cryptococcus Neoformans*

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Cryptococcus neoformans is an encapsulated opportunistic fungal pathogen that annually kills hundreds of thousands individuals worldwide. Infection results in high morbidity and mortality, even with expensive and toxic antifungal treatments. After inhalation of infectious particles, *C. neoformans* undergoes a series of transcriptional and regulatory responses that initiate adaptation to the host environment and production of virulence factors. One key player in this adaptation is the Cyclin-dependent kinase 8/Cyclin C system, which has been studied in the model yeast *Saccharomyces cerevisiae* and in humans, but not in the context of a pathogenic fungus. The component proteins specifically regulate transcription by RNA polymerase II through two mechanisms: they can inhibit the assembly of the Mediator subunit, which subsequently inhibits assembly of the RNA Pol II holoenzyme. Cdk8 independently can also phosphorylate the carboxy-terminal domain of RNA Pol II polymerase, which also inhibits transcription. It has also been reported that the Cdk8/Cyclin C system directly influences mitochondrial morphology and function by promoting mitochondrial fragmentation. In *C. neoformans*, deletion of the genes encoding Cdk8 or Cyclin C results in severe defects in oxidative stress resistance and attenuated virulence. Deletions also yield abnormal mitochondrial morphologies, both at rest and upon oxidative stress. In related species of *Cryptococcus*, mitochondrial morphology plays a key role in enhancing fungal survival and pathogenesis in the mammalian host environment. Despite these intriguing phenotypes and clear relevance to human disease, little is known about how Cdk8/Cyclin C influence gene regulation and mitochondrial function in this fungal pathogen. To understand these phenotypes and relate them to virulence, I am investigating the molecular mechanisms by which this system controls cryptococcal mitochondrial morphology and virulence factors.

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ALR protein, a Critical Protein in Cardiac Development, Regulates Cellular Iron Homeostasis by Altering Mitochondrial Import of ATP-binding Cassette (ABC)-B8

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Iron is an essential molecule for normal cellular physiology, and altered cellular iron homeostasis is commonly observed in diseases with disruption of iron/sulfur (Fe/S) cluster maturation, such as cardiomyopathy associated with Friedreich's ataxia. Inhibition of Augmenter of Liver Regeneration (ALR), a mitochondrial inter-membrane-space protein involved in mitochondrial protein import, results in cardiac developmental defect in zebrafish, and its mutation 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. Using a knockdown-rescue approach, we further demonstrated that only the mitochondrial, but not the cytosolic, ALR isoform is involved in 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 clusters. Downregulation of ALR reduced the mitochondrial levels of ABCB8, while ABCB7 levels were not affected. We also identified defects in mitochondrial import of ABCB8 peptide as the mechanism for reduced mitochondrial ABCB8 levels with ALR knockdown. Finally, we demonstrated that ABCB8 physically interacts with the protein import system consisting of ALR and Mia40, thus providing a mechanism for reduced ABCB8 mitochondrial transport with ALR downregulation. **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 and cardiac development.

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The Intestinal Stem Cell Niche is Intrinsically Drug Resistant

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The epithelium of the small intestine plays an essential role in the uptake of dietary nutrients while simultaneously providing a barrier against xenobiotics and microbial metabolites present in the gut lumen. In part to cope with the exposure to diverse toxins, this epithelium is one of the most rapidly proliferating tissues in the body, capable of completely turning over in 3 – 5 days. The stem cells responsible for this proliferative capacity reside at the base of the crypts of Lieberkühn, regulated by a stem cell niche comprised of both epithelial and stromal components. This niche provides a protective structural environment as well as a milieu of signaling factors required for self-renewal, proliferation and differentiation. Key signaling factors responsible for intestinal stem cell homeostasis such as Wnts and R-Spondins are produced by subepithelial myofibroblasts in the stroma of the niche. Wnt production from all cells, including the stromal myofibroblasts, requires PORCN activity. PORCN is an O-acyl transferase in the endoplasmic reticulum that post-translationally palmitoleates Wnts, a modification required for Wnt secretion and receptor binding. It was therefore unexpected that oral administration of potent orally bioavailable PORCN inhibitors, such as ETC159 and Wnt-C59, are effective in shrinking Wnt-driven cancers but do not affect intestinal homeostasis at therapeutically effective doses. Conversely, Wnt pathway inhibitors such as DKK1 and XAV939 that block signaling directly in intestinal epithelial stem cells are highly toxic to the intestine. This led us to hypothesize that the stromal compartment of the intestinal stem cell niche is intrinsically drug resistant. Here we test that hypothesis. We find that co-culture with stromal myofibroblasts confers resistance to PORCN inhibitors in cultured intestinal organoids ex vivo. Comparing the transcriptomes of intestinal stroma versus epithelium, we determine that a subset of ATP-binding cassette (ABC) proteins that encode transporters are highly expressed in the stromal cells. In line with this, a broad-spectrum inhibitor of efflux pumps is able to block the in vitro efflux of a number of compounds by myofibroblasts. We thus conclude that intrinsic drug and xenobiotic resistance is a unique property of the myofibroblasts of the intestinal stem cell niche that preserves stem cell function and normal tissue homeostasis in the face of an unpredictable environment.

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BVES Loss Delays Colonic Regeneration After Mechanical Injury

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Bves is a tight junction associated protein that regulates cell adhesion associated signaling and epithelial to mesenchymal transition (EMT). Based on these observations, we hypothesized that *Bves* contributes to junctional integrity and wound resolution after colonic injury. **Methods:** We employed a mechanical injury model using an endoscope with a port for biopsy forceps. 4 biopsies were taken at a fixed distance from the rectum in a proximal to distal pattern in wild type (WT, n =22) and *Bves*$^{-/-}$ mice (n=24). We repeated endoscopy 2,4, and 6 days post-injury to quantify wound closure. Ultrastructural differences were evaluated by electron microscopy. Permeability between *Bves*$^{-/-}$ and WT colon was assessed using a novel 3D enteroid culture model and an ex vivo model employing Ussing Chambers. Immunofluorescence was used to characterize proliferation and neutrophil recruitment in the wound and peri-wound environment. Crypt fission was calculated by performing crypt isolation and comparing the number of branching crypts between WT and *Bves*$^{-/-}$colon. **Results:** 6 days post-injury, 74% of *Bves-/-* mice survived as compared with 100% of WT mice (P = 0.015), suggestive of wound persistence. Wound area was not different in *Bves-/-* versus WT mice at day 2 or day 4; however, wound area was significantly greater in *Bves-/-* mice at day 6 (21,380 ± 4,062 pixels versus 6,654 ± 1,784 pixels, P < 0.001). There were fewer neutrophils in the peri-wound and wound area in *Bves*$^{-/-}$ mice as compared with WT at both 4 hours (44.75 ± 32.16 vs. 249.0 ± 178.1 P = 0.029, n=5) and 6 days (2.4 ± 0.75 vs. 14.4 ± 4.1, P = 0.021, n=5) after injury. There were also a fewer number of pH3 positive cells/crypt in *Bves*$^{-/-}$ mice as compared with WT in the wound and peri-wound area 4 hours (19.25 ± 4.13

vs. 48.75 ± 10.89, P = 0.045, n=5) and 6 days (27.4 ± 6.19 vs. 57.80 ± 6.32, P = 0.009, n=5) after injury. Crypt fission was decreased in *Bves*$^{-/-}$ mice as compared with WT mice 4 hours (0.91 ± 0.02% vs. 4.03 ± 0.09%, P = 0.042) and 3 days (0.008 ± 0.016% vs. 6.19 ± 0.04%, P < 0.0001) after colonic injury. Electron microscopy revealed dilated intercellular spaces in the *Bves*$^{-/-}$ mouse colon (82 ± 16 nm versus 41 ± 2 nm, P = 0.012) and there was increased permeability in the *Bves*$^{-/-}$colon by both transepithelial resistance in an enteroid model (211.9 ± 5.57 ohms vs. 267.4 ± 19.5 ohms, P < 0.001, n=3) and by FITC-dextran penetration in an *ex-vivo* model (0.020 ± 0.003 mg/mL vs. 0.003 ± 0.001mg/mL, P = 0.002, n=4). **Conclusion:** *Bves* contributes to junctional integrity and resolution in colonic wound healing.

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Population Segmentation Based on Healthcare Needs: A Systematic Review

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Context: Population segmentation based on healthcare needs is a promising approach for enabling the development and evaluation of healthcare service models to meet healthcare needs. In order to identify an instrument which operationalizes the 'Bridges to Health' model of population segmentation, we conduct a systematic review for adult population healthcare need based segmentation tools. **Methods**: Using search terms reflecting concepts of population, healthcare need, and segmentation, we obtained 8256 articles from PubMed, CINAHL and Web of Science databases after removing duplicates. We included segmentation tools for adult populations with mutually exclusive segments while requiring different segments within a segmentation framework to offer prognostic value for clinically relevant outcomes. All shortlisted articles were hand searched and relevant data e.g. validation studies extracted. **Findings**: We identified a total of 11 unique adult population healthcare need based segmentation tools. Validated tools include proprietary instruments such as the Johns Hopkins' Adjusted Clinical Groups (ACG), 3M's Clinical Risk Groups, and Kaiser Permanente's Senior Segmentation Algorithm. Meanwhile, validated non-proprietary tools included 3 tools formulated from elderly patient datasets in Canada, Netherlands and Taiwan respectively. Finally, non-validated tools included Lombardy, North-West London and Delaware population segmentation schemes, Complexedex and 'Bridges to Health'. The underlying segmentation bases of most identified tools were found to be conceptually comparable to each other. We found no validated tools designed to operationalize the 'Bridge to Health' model. **Conclusions**: Adult population healthcare need based segmentation tools can be categorized based on whether they are validated, require comprehensive electronic medical records, proprietary status, and number of segments. Evaluating tools in this manner allows policy makers to identify the most optimal tool for adaptation in their respective healthcare systems. Future work include further validation, comparative evaluation of conceptually similar segmentation tools, as well as development and evaluation of healthcare service packages tailored for specific population segments.

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Imaging the Extravascular Space using Acoustically Activated Nanodroplets

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Background. Heart disease remains the leading cause of death in the United States, and myocardial infarctions (MI) are the number one killer. Prompt diagnosis and treatment of MIs leads to the best patient outcomes. Ultrasound contrast agents (microbubbles or MB) have revolutionized cardiac diagnostic imaging. MBs have been utilized within the intravascular space to quickly determine perfusion and to look at cardiac wall abnormalities. Commercially available MBs, such as Definity, contain perfluorocarbon gas encapsulated by a lipid shell. MBs can be compressed creating nanodroplets (ND), which are sub-micron particles. These particles can be reactivated and imaged using diagnostic ultrasound. In this study, we used NDs to determine whether extravascular spaces within cardiac tissue can be imaged with diagnostic ultrasound. **Methods**. Transthoracic studies were performed in a rat myocardial ischemia/reperfusion model. These rats experienced acute myocardial infarction via left anterior descending artery (LAD) ligation followed by reperfusion at 45 minutes. After 48-72 hours, the animals underwent transthoracic imaging with a Siemens 15L8 transducer (Siemens Acuson Sequoia; Siemens Healthcare, Erlangen, Germany) utilizing contrast pulse sequencing (CPS). Two hundred microliter injections of Definity MBs were administered during both low-mechanical index (0.5) and high-mechanical index (1.5) imaging at one frame every four cardiac cycles during and at periodic intervals after the injection. Injections of Definity ND were administered in the same form. During imaging, droplet activation was monitored in the normal and infarct zones. After these injections, the animals were euthanized, and serial cross sections of the left ventricle were stained with triphenyltetrazolium chloride (TTC). **Results**. Two imaging techniques were utilized to detect the Definity NDs within the infarct zone. When low-mechanical index imaging was used, the images showed no contrast. However, two minutes post-injection, high-mechanical index imaging was resumed, and contrast was observed within the infarct zone and the LV cavity. In subsequent frames, contrast through most of the heart was cleared leaving only enhancement within the infarct zone. The area of contrast enhancement corresponded to the area of infarction confirmed with TTC staining. **Conclusions**. Activation of Definity ND within infarct zones was possible with a diagnostic, commercially-available ultrasound system. This methodology of targeted real-time imaging may improve detection of myocardial ischemia and be an alternative diagnostic test for MI.

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Evidence of Fetus Associated Microbiota and its Potential Maternal Origins in a Non-human Primate Model of Maternal High-fat Diet and Obesity.

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The trillions of commensal bacteria that exist within our bodies, collectively known as our microbiome, are major contributors to host metabolism and are essential for directing proper immune and neurologic development. Understanding when and how an infant acquires its full repertoire of microbiota in early life will provide insight into critical host-microbial interactions that either promote health, or contribute to disease that manifests later in life, such as atopic disease, auto-immune disorders and obesity. The neonate is assumed to be first colonized by bacteria immediately after birth, but evidence of bacteria associated with the placenta and aminotic fluid of healthy, term pregnancies is challenging the presumption of a sterile intrauterine environment. However, how microbiota can colonize this space and its relative effect on microbiome development in the neonate is unknown. To explore the possibility of fetal associated bacteria in gestation, we sought to characterize the fetal microbiota in a non-human primate model of high-fat diet and obesity, determine its maternal origin, and evaluate the impact of a high-fat diet on microbial colonization of the fetus. **Methods:** Japanese macaque dams were fed a control or high-fat diet (13 vs. 36% fat) during pregnancy and nursing. At gestational day 130 (G130), equivalent to 32 weeks gestational age in humans, offspring were delivered by Cesarean (n=5). Swabs of the fetal colon and oral cavity were obtained, along with samples of the maternal anus, oral cavity, placenta and vagina. Anal swabs separate diet-matched juveniles were additionally obtained at 6 to 10 months of age for postnatal comparisons (n=7). 16S rRNA gene sequencing was performed with negative kit and sequencing controls to interrogate the microbiota within the samples. Sequencing data was analyzed using in-house computational pipelines to filter taxa present in sequencing controls and generate a relative abundance table of identified taxa. **Results:** The fetal colon and oral cavity harbored a distinct microbial community at G130 distinct from most maternal body sites, but largely similar in community structure and composition to the placenta by Bray-Curtis distance measures. Prediction of the maternal origin of the fetal microbiota using a Bayesian approach identified the placenta microbiota as the predominant and most likely source. Comparisons between offspring exposed to a maternal high-fat or control diet during gestation revealed distinct differences in the fetal gut microbiome by virtue of diet that persisted into postnatal life. **Conclusions:** This data supports the notion of a normally non-sterile intrauterine environment in pregnancy. It further suggests that fetal colonization by microbes may be initiated as early as 32 weeks gestation, which in large part originates from the placenta. Lastly, this process appears to be modulated by maternal diet in pregnancy, further underscoring the importance of maternal gestational nutrition to long-term infant health.

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Investigating the Role of the Tumor Microenvironment in Resistance of Urothelial Carcinoma of the Bladder to Cisplatin Therapy

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Urothelial carcinoma of the bladder is the sixth most prevalent cancer in the United States with an estimated 75,000 new cases and 16,000 deaths each year. Bladder cancer recurrence remains common and is a significant contributor to patient morbidity and mortality. Many patients, who initially respond to cisplatin as part of the standard of care treatment, exhibit refractory disease within a few years, or have resistance to therapy upon diagnosis. Recent studies have demonstrated that the tumor microenvironment plays a role in resistance to therapy in many cancer types, including bladder cancer. The overall objective of this study was to identify the constituents of the tumor microenvironment that confer resistance to cisplatin therapy in bladder cancer using MicroEnvironment MicroArrays (MEMA), which consists of robotically printed growth pads made up of combinations of functional extracellular matrix (ECM) components, growth factors and cytokines found in different local and metastatic microenvironments. The goal was to use a cisplatin-sensitive bladder cancer cell line, RT112 cells, in the MEMA screen to identify candidate microenvironment components that increased cellular survival and proliferation upon cisplatin treatment. The MEMA results identified candidate microenvironment constituents that confer resistance to cisplatin. Of these candidates, lymphatic vessel endothelial hyaluronic acid receptor 1 (LYVE-1), a CD-44 homologue, potently induced resistance to cisplatin. Interestingly, when RT112 cells were grown on high or low molecular weight hyaluronic acid growth pads there was differential resistance to cisplatin incited by LYVE-1. Currently, the mechanisms involved in LYVE-1/hyaluronic acid-mediated resistance to cisplatin therapy in bladder cancer are being elucidated. Future work will be aimed at inhibiting the interaction of LYVE-1 with bladder cancer cells to rescue sensitivity to cisplatin. Characterizing the mechanisms of resistance associated with the tumor microenvironment upon cisplatin therapy will lay the foundation for improved treatment options and better overall patient outcomes. Results from this work have the potential to provide new information that will enable us to devise novel targeted approaches aimed at anticipating or overcoming resistance to cisplatin therapy in bladder cancer.

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Birthing Choices in Southern Mexico: Perspectives on the Options and Issues

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Non-emergent Cesarean sections (C-sections) pose a challenge to public health systems worldwide, incurring additional costs and increasing risk of complication for mothers and infants. Mexico maintains one of the highest C- section rates in the world, far surpassing the World Health Organization's recommended national C-section rate. Although private institutions in Mexico have historically possessed the highest rates of C-sections, public hospitals have recently experienced a surge in C-sections. In contrast to patients in private hospitals that directly elect and pay for C-sections, patients in public hospitals receive care subsidized by the

government, limiting their options for selecting physicians, health care facilities, and services. While previous studies have analyzed the process of physician and patient decision-making regarding C-sections in private hospitals, the increasing C-section rate in public hospitals remains poorly understood. This qualitative study examines physicians' decision-making regarding delivery method at two public hospitals in Southern Mexico. The methodology of this study includes 27 semi-structured interviews with resident and attending physicians and over 120 hours of observation in the labor and delivery ward. Preliminary findings in this study reveal three critical influences on physician decision-making: the dynamics of medical hierarchy, the impact of institutional structure, and clinicians' perceptions of patient characteristics. The results of this study can provide greater insight as to the increased C-section rate in public health institutions, potentially decreasing the financial burden of non-emergent C-sections and improving maternal and infant well-being.

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An Enhanced Live Attenuated Influenza Vaccine Does Not Elicit an Innate Inflammatory Infiltrate yet Still Protects Against H7N9 Challenge

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The live attenuated influenza vaccine (LAIV) is preferentially recommended for use in children aged 2-8 in Europe, but is considered insufficiently safe for the most at risk groups (including infants and very young children), because of a modest propensity to induce wheezing. In pursuit of a safer vaccine for these groups, we previously developed an experimental animal (mouse) model to study the safety and immunogenicity of LAIV, by introducing the attenuating mutations of LAIV into the genetic background of the mouse-adapted, highly virulent Influenza A Virus (IAV) strain, A/PR/8/34 (PR8). This resulted in a modified LAIV that retained virulence in C57/BL6 mice, such that it is possible to identify additional attenuating mutations that further reduce the pathogenicity of this virus. Additional work showed that adding a mutation at residue 319 of PB1 conveys a dramatic safety increase with only a modest decrease in immunogenicity. This virus (LAIV-PB1-319Q) therefore represents a potential prototype for an enhanced LAIV (eLAIV). Our previous work suggested that neutralizing antibodies were dispensable for LAIV-mediated protection against challenge with a lethal dose of heterologous virus. In the present work, we tested the ability of eLAIV (LAIV-PB1-319Q) to protect mice against challenge with an antigenically distinct pandemic H1N1 virus and a heterologous H7N9 virus, and found that eLAIV elicited protection against the H7N9 challenge virus, in the absence of neutralizing antibodies. We further showed that while both eLAIV and LAIV elicited influenza-specific T cells within lung tissue, intranasal vaccination with LAIV (but not eLAIV) induced robust innate immune cell accumulation in the lung – suggesting that eLAIV may be less prone to trigger lung inflammation than conventional LAIV. These findings have important implications for the development of an improved, live attenuated influenza vaccine.

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Monoacylglycerol Lipase Inhibitors Produce Antinociception through Cannabinoid Receptor 1- and 2-dependent Manners in the Mouse Paclitaxel Model of Chemotherapy-induced Peripheral Neuropathy

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Chemotherapy-induced peripheral neuropathy (CIPN), manifesting as burning, tingling or numbness in the hands and feet, represents a serious side-effect caused by a variety of anti-neoplastic agents, such as paclitaxel. CIPN often requires a dose-reduction or cessation of use, deviating from established protocols for cancer treatment. Because of there are presently no effective preventative or analgesic strategies available for the management of paclitaxel-induced peripheral neuropathies, a serious need exists for the identification of novel antinociceptive agents. The endogenous cannabinoid system contains a variety of therapeutic targets for the management of paclitaxel-induced neuropathies. Direct cannabinoid binding (CB) receptors 1 and 2 agonists reduced mechanical allodynia, defined as a nociceptive response to non-nociceptive light touch, in rodent models of paclitaxel CIPN. Here, we tested the hypothesis that inhibition of monoacylglycerol lipase (MAGL), the primary degradative enzyme of the endogenous cannabinoid 2-arachidonoylglycerol (2-AG), will reverse paclitaxel-induced mechanical allodynia. Four intraperitoneal (i.p.) injections of paclitaxel (8 mg/kg) given every other day to C57Bl/6J mice produced a significant and long-lasting mechanical allodynia as assessed using von Frey filaments. The selective and potent MAGL inhibitors, JZL184 (1.6-40 mg/kg) and MJN110 (1-5 mg/kg; i.p.), reversed mechanical allodynia in paclitaxel-treated mice to pre-paclitaxel thresholds in time- and dose-dependent manners. Maximum anti-allodynic effects occurred two and three hours post-administration, respectively, with ED_{50} values (95% confidence limits) of 12.2 (7.5-19.9) and 2.0 (1.1-3.5) mg/kg. MJN110 was 6.1 (2.8-13.6) fold more potent than JZL184. Furthermore, complementary pharmacologic and genetic approaches demonstrated that the anti-allodynic effects of both inhibitors required both CB_1 and CB_2 receptors . Ongoing studies are examining markers of spinal inflammation associated with paclitaxel administration (e.g., astrocyte activation, microglia activation, and pro-inflammatory cytokine production) to test the hypothesis that MAGL inhibition will decrease spinal inflammation in paclitaxel-treated mice. The results of the present study suggest that MAGL represents a viable pharmacologic target for the treatment of CIPN.

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Development of a Thumb Carpometacarpal Joint Force Application Device

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Osteoarthritis (OA) of the thumb carpometacarpal (CMC) joint affects ~50% U.S. adults over 55, causing pain and functional deficits while performing commons tasks like opening jars and medications. While the symptoms of OA are characterized by a restricted range of motion and waning grip strength, minimal research has investigated the loss of individual muscle actions that correlate to this reduced function. We hypothesize that loss of grip strength associated with OA is directly related to asymmetric decline in magnitude and direction of force applied by the thumb CMC joint, and that characterization of this decline will provide insight into OA pathophysiology. However, no device is currently capable of measuring the applied force of the thumb CMC joint during each motion. In this study, we built a thumb joint apparatus that attaches to a multi-axis load capable of measuring forces applied during flexion (palmar abduction), extension, (radial) abduction, and adduction. The apparatus was built to meet the following objectives: 1) adjustable to accommodate varying hand size, 2) hand placement is consistent and measures are repeatable, 3) isolates thumb CMC joint action, 4) can detect force magnitude and direction, and 5) is durable to accommodate numerous patients. Preliminary testing showed that force application during thumb CMC joint motions is best isolated when the hand has neutral wrist positioning and has support on the ulnar and palmar sides of the hand. Incorporating our preliminary results with our objectives, we machined a palmar hand support of aluminum and a connected structure that allowed for mounting of the load cell. Both the palmar hand support and load cell base are adjustable to accommodate varying hand sizes. Thumb forces are applied to a customized circular ring that is connected to the load cell. The circular ring allows for uniform and symmetrical thumb movements to accommodate bilateral thumb testing. Forces can be measured for each thumb CMC joint action independently. Additionally, the entire apparatus is portable, and can be used to test subjects in a variety of locations. The system is designed to be placed on an adjustable table top, and used along with a standard adjustable office chair. Pilot testing suggests that our apparatus can be used with both OA and asymptomatic subjects. Testing of 5 healthy participants on multiple days also indicated that our unique system provides consistent positioning of the hand during thumb CMC joint flexion, extension, abduction, and adduction, resulting in ~15% variability in force measurements between test days. Phase two testing will explore our devices capacity to detect functional variation between asymptomatic and OA subjects prior to and after completion of a thumb CMC joint specific exercise regimen.

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Modeling Phase 1 Reactions: Towards Metabolite Prediction

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Metabolism has great implication on the absorption, efficacy, excretion and toxicity profiles of drugs. While current computational metabolism models can rapidly and cost-effectively predict the sites of metabolism on thousands drug candidates, they are unable to predict what specific metabolic transformations would happen at certain sites and therefore what metabolites would be formed. Without knowledge of potential metabolite structures, it is impossible for pharmaceutical chemists to differentiate harmful metabolic transformations from beneficial ones. In this research, we model the majority of competing phase I reactions observed in human and human tissues using machine learning. We group these reactions into five classes: stable oxygenation, unstable oxygenation, dehydrogenation, hydrolysis, and reduction based on their mechanisms. Reactions in each of these classes transform the parent compound at the site of metabolism in the same way. We train a multi-target neural network model on literature derived data and assess the model performance using various accuracy metrics. Among 4391 unique human substrates, our method identifies correctly the class-specific site of metabolism in each molecule as top1 or 2 prediction with, respectively, 80.3% and 88.6% accuracy. Among 450,838 potential sites, our model identifies correct class of biotransformation with 97% area under the receiver operator curve.

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Optimizing Hematopoietic Stem Cell Reprogramming in Adult Human Fibroblasts

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Our current inability to culture hematopoietic stem cells (HSCs) *in vitro* remains a significant problem in the study of hematopoiesis. The scarcity of viable patient specific cells for *in vitro* research, as well as therapeutic transplants, hampers the study of various hematologic pathologies and drug screening efforts to treat these disorders. To obtain sufficient numbers of HSCs both *in vitro* and *de novo,* recent studies focus on reprogramming pluripotent stem cells or somatic cells, with most success found in studies that mimic developmental hematopoiesis. We recently demonstrated our ability to induce a hemogenic program in mouse embryonic fibroblasts through overexpression of a minimal set of transcription factors (TFs) – Gata2, Gfi1b, and cFos (GGF). Cells reprogrammed with these factors traverse an endothelial intermediate that then gives rise to $CD45^{+}$ hematopoietic cells upon prolonged culture. We are now extending this strategy to the human system. Optimization of hemogenic induction in adult human fibroblasts would allow creation of patient- and disease-specific hematopoietic cells for eventual HSC transplants, as well as *in vitro* disease modeling and drug testing platforms. Although we show some success in reprogramming human fibroblasts into HSC-like cells with the same TFs we used in the mouse system, improvement of their yield and function is still required. Screening of various co-culture systems

identified OP9-DL1 and OP9-DL4 as hematopoiesis supporting stroma that impart functional potential on reprogrammed cells. Reprogrammed cells on OP9-DL1 generate multilineage colonies in colony forming unit assays. OP9-DL4 co-culture permitted the formation of cobblestone areas after long-term culture, indicating the acquisition of hematopoietic function. We have also identified an additional TF – GFI1 – that improves the yield and function of the resulting HSC-like cells (the new TF cocktail is termed 3GF). Analysis of $CD49f^+$, $CD49f^+CD34^+CD90^+$, $CD49f^+CD34^+CD90^+BB9^+$, and $CD49f^+CD34^+CD90^+BB9^+CD45^+$ cells demonstrate significant increases to all populations with the addition of GFI1 to the TF cocktail. $CD45^+$ populations emerge in both GGF and 3GF reprogrammed cells on day 35 of culture, demonstrating the development of hematopoietic cells *in vitro*. These results together demonstrate our improvements to human hemogenic induction, bringing us a step closer to applying these findings to translational medicine.

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A Role for Interleukin-2 Signaling in Impaired Wound Healing in Systemic Lupus Erythematosus

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Systemic lupus erythematosus (SLE) is an autoimmune disease in which overactivation of the immune system damages many organs of the body. Notably, skin lesions, including excessive scarring and impaired wound healing, are common in SLE. Interleukin-2 (IL-2), an immunologic signaling molecule known to be dysregulated in SLE, may play a role in the wound healing impairment that occurs in individuals with SLE. Quercetin, a naturally-occurring chemical found in tea and berries, is known to alter IL-2 signaling and may represent a method for further exploring the relationship between IL-2 and wound healing in SLE. The purpose of this study was to determine the role of IL-2 in the mechanism underlying the wound healing deficit in SLE. **Methods:** The B6.MRL-*Fas*^{lpr}/J mouse strain served as the SLE model. All animal procedures were approved by the Institutional Animal Care and Use Committee. Both SLE-model and wild-type (WT) mice were wounded with a biopsy punch and treated with quercetin or control. Wound healing progress was monitored by measurement with calipers. The expression of IL-2, its receptor components, and its downstream signaling pathway, were explored using immunohistochemistry (IHC) and western blotting (WB). **Results:** SLE-model mice heal at a normal rate compared with wild-type (WT) mice and quercetin treatment delays wound healing only in the SLE model. Immunohistochemistry demonstrates an increase in skin and especially wound IL-2 accumulation and IL-2 receptor subunit expression in SLE-model mice compared with WT. Quercetin treatment did not appear to alter skin or wound IL-2 expression in the WT. However, quercetin did appear to alter IL-2 receptor component levels in the SLE-model mice. IL-2 receptor-α increased in quercetin-treated SLE model mice compared with vehicle-treatment in the SLE model while IL-2 receptors-β and γ decreased. Furthermore, downstream signaling via the Janus Kinase-1 (Jak-1) and Akt pathways was diminished in the wounds and skin only in the quercetin-treated SLE-model mice. **Conclusion:** The use of quercetin, an anti-fibrotic agent, offers a novel approach to exploring the wound healing deficit seen in SLE in a model of SLE. It appears that in the context of an SLE-model, a decrease in signaling downstream of IL-2 induced by quercetin may impair wound healing. Decreased IL-2 signaling may therefore underlie the impaired wound healing in SLE. IL-2 represents an attractive treatment target for wound healing impairment in SLE and therefore warrants further exploration. *Conflicts of interest: The authors have no relevant conflicts of interest to disclose.*

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Enhancing the Potential of Cell Based Therapeutics through the Development of a Tunable, Orthogonal Signal Processing Network in Mammalian Cells

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Cell based therapies are an emerging class of novel therapeutics, particularly promising for cancer and autoimmune diseases. As the conditions we aspire to target and treat with this new methodology become more complex, this will require cells to recognize several environmental markers to differentiate healthy from diseased tissue. To this end, engineered cells will need intracellular machinery capable of integrating multiple signals to induce conditional therapeutic gene expression. We report here on the successful adaptation of zinc finger based synthetic transcription factors (sTFs) to attain these goals in mammalian cells. The system comprises multiple orthogonal activating sTFs and several classes of inhibitory sTFs that act on a library of sTF-responsive, engineered promoters. **Methods:** Plasmids encoding constitutively expressed sTFs or synthetic biosensors and their cognate promoters were transiently transfected into HEK293FT cells. When activated, promoters expressed enhanced yellow fluorescent protein (EYFP) as a reporter gene, which was analyzed with flow cytometry. **Results:** sTF-responsive, engineered promoter strengths spanned three orders of magnitude, such that the output of the system is readily tunable to allow for a simple adjustment of the dose of a secreted therapeutic. Next, promoters that require two and three activating sTFs to achieve full gene expression and that can be repressed by inhibitory sTFs were designed and tested, allowing cells to perform logical evaluation of multiple cues. Cellular sensitivity to two competing signals was tuned by altering the DNA-binding affinity of each sTF. Furthermore, as modifying the DNA-binding affinity of activating sTFs creates unique dose response profiles for each, this provides a strategy to wire existing synthetic biosensors into the system – by matching the required sensitivity of the receptor to an appropriately sensitive (or insensitive) sTF, further rounds of receptor engineering, a time-consuming process, can be avoided. **Conclusion:** In summary, our discoveries have enabled cells to integrate multiple signals with technology that is readily tunable at several points and can be tailored to receive inputs from synthetic biosensors. Furthermore, the outputs from this system are readily adaptable to proteins of therapeutic interest, such as stimulatory cytokines in tumors and suppressive cytokines in autoimmune inflammation. We are additionally leveraging this new biochemical tool set for applications including programmable engineered cell-based therapies as well as customizable gene expression programs to interrogate development and drive differentiation.

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Regulation of the Adipose Peroxisome Proliferator-Activated Receptor γ - Fatty Acid Binding Protein 4 - Uncoupling Protein 2 Axis and Reduction of Oxidative Stress After Bariatric Surgery

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Despite advances in medical management, bariatric surgery (BS) remains the most effective treatment for weight loss and type 2 diabetes mellitus (T2DM). However, a detailed mechanistic understanding of how BS is able to reverse some of the "metabolic syndrome" phenotype even before significant weight loss is achieved remains undefined. One target of interest has been Peroxisome Proliferator-Activated Receptor γ (PPARγ) whose activity modulates whole body insulin sensitivity. Here we report results of an axis whereby how BS can function to reduce oxidative stress within adipose tissue. BS modulates PPARγ expression, leading to a decrease in fatty acid binding protein 4 (FABP4) in white adipose tissue. FABP4 suppression occurs conjointly with increased uncoupling protein 2 (UCP2), leading to a reduction in measures of white adipose tissue oxidative stress as assessed by cysteine oxidation. This axis may function as one of the early mechanisms by which BS reduces the metabolic syndrome phenotype.

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Usefulness of Computed tomography Coronary Calcium Score to Predict Coronary Artery Disease Among Patients Evaluated for Liver Transplantation

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Background & Aims: Incidence of coronary artery disease (CAD) is 2- to 3-fold higher in liver transplant (LT) patients, and cardiovascular disease is the most important non-hepatic cause of death in this population. To date, there is no consensus on the preferred screening tests to detect CAD in LT candidates, and a low sensitivity for cardiac stress testing has been reported. Coronary calcium score (CCS), as determined by CT scan, is a useful alternative to investigate risk for CAD. Our aim was to investigate the usefulness of CCS to detect CAD, along with other commonly used tests or risk factors in patients undergoing LT evaluation. **Methods:** All patients evaluated for LT between 2014 and Aug/2016 were included. Apart from CCS, cardiovascular risk factors were obtained in all cases. Cardiac stress testing with nuclear medicine (NM) or dobutamine stress echocardiogram (DSE) were compared to CCS >400 as predictors of significant CAD (>50% stenosis) found during coronary angiography (CA). **Results:** We included 79 LT evaluations, mean age 58±8, male in 59%. Mean BMI was 29±5 kg/m^2 and MELD 19±7. Most common etiologies were viral hepatitis (33%), NASH (25%), alcohol (23%), and autoimmune liver disease (13%). CAD risk factors were: poor functional capacity in 80%, smoking (20 pack-year) in 39%, diabetes mellitus in 27%, prior/current hypertension in 25%, dyslipidemia in 16%, prior CAD or stroke in 14%, and chronic kidney disease in 10%. Median CCS score was 76 (0.5-587), and it was >400 in 25 (32%). NM or DSE was performed in 50 (63%) with a positive result in 5 (8%). A CA was performed in 35 patients, and it revealed CAD in 15 (43%). All patients with CAD were asymptomatic. Sensitivity and specificity for detecting CAD were 50% and 69% for CCS, 25% and 73% for NM/DSE, 43% and 57% for presence of >3 cardiovascular risk factors, and 100% and 67% for a troponin I >0.07, respectively. In patients with both a positive CCS and risk factors, only a marginal improvement in sensitivity (60%) and specificity (67%) was noted. However, having either a positive CCS or an elevated troponin I improved diagnostic accuracy for CAD (sensitivity 60%, specificity 100%). **Conclusions:** CCS was the most useful single predictor of significant CAD in asymptomatic patients undergoing LT evaluation. The usefulness of cardiac stress testing in this clinical scenario was rather poor. CCS >400 or a troponin I >0.07 was the most accurate combination to detect asymptomatic CAD in LT candidates.

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The Role of the Receptor Tyrosine Kinase Flt3 in Dendritic Cell Development

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Flt3 is a receptor tyrosine kinase known to be involved in dendritic cell development. However, the extent of dendritic cell deficiency in Flt3 knockout mice is not as severe as in the knockout for the ligand of this receptor, Flt3L. This suggests that Flt3L may bind to a second compensatory receptor in order to support dendritic cell development and that this may identify a noncanonical pathway for their development. Using in vitro and in vivo methods we have demonstrated that Flt3L does not appear to bind to a secondary receptor but instead that loss of Flt3 impacts the development of bone marrow progenitor cells that give rise to dendritic cells. Absence of Flt3 is dominant over absence of Flt3L such that double knockout mice have dendritic cell development levels comparable to Flt3 knockout mice. The increase in bone marrow progenitors seen in Flt3 knockout mice is sufficient to enable to development of dendritic cells in a non-Flt3 dependent manner and these dendritic cells are fully functional and capable of performing their specialized functions. These dendritic cells are also transcriptionally similar to those that develop in wildtype mice. We have thus identified a noncanonical pathway for dendritic cell development that does not require the receptor Flt3 but which still generates dendritic cells with full functional capabilities. This suggests that hematopoietic development is not strictly dependent on classically defined developmental pathways and that cytokines may play supportive rather than instructive roles in cell differentiation.

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HCV Genie: A Web 2.0 Interpretation and Analytics Platform for the Versant Hepatitis C Virus (HCV) Genotype Line Probe Assay Version 2.0

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Hepatitis C virus (HCV) genotyping at our institution is performed using the Versant Hepatitis C virus genotype 2.0 Line Probe Assay (LiPA). The last step of this procedure is a manual, laborious, and error prone process that involves the comparison of band on a test strip to a physical reference table. We first developed a web-based HCV genotype interpretation platform that would (a) minimize interpretation time, (b) reduce error, and therefore (c) increase the quality of patient care delivered through this test methodology. The original HCV Genie was written, deployed, clinically validated, and proven to be identical to human expert interpretation (n = 200) over the course of 2 weeks. It decreased the time needed to interpret results by 53% in residents, but results among experienced lab technicians are more equivocal.

As a follow-up to this work the database was ported to a pure web environment and a novel image analysis step was added. This allows an institution to utilize a scanned LiPA image to directly generate the genotyping results. **Methods:** The image analysis step make use of common algorithms ported to the web environments such as Sobel edge detection, and use a novel windowed Hough transform to detect bands and band position. The program utilizes Github gh-pages as a web server and employs the client side JavaScript as the programming language. This allows analysis to be performed without downloads, or potential patient data leaving the computer the analysis is performed on. **Conclusions:** Our original program provided results that are identical to the manual workflow, but (a) with reduced manual steps and (b) in a timeframe similar to that of the well-trained manual interpreter, regardless of the program user's experience level. This iteration focused on developing lane and band detection algorithms, and creating a publically available tool that eliminates data privacy concerns. Future iterations of this program will focus on allowing additional user input, and add the ability to store and aggregate results in a database of their choosing with ease, allowing for advanced data analytics of HCV genotypes.

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Data-driven Motor Subtypes of Early Parkinson's Disease

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Objective: To identify and characterize subtypes of Parkinson's disease (PD) in newly diagnosed patients using data-driven hierarchical clustering approaches. **Background**: It is widely accepted that PD is a heterogeneous disease with multiple clinical presentations. Empirically recognized motor subtypes of PD include a tremor dominant (TD) group and a postural instability and gait disorder (PIGD) group. Some clinicians also recognize an ill-defined intermediate group. However, subtyping assignments are primarily based on clinical observation of motor features. **Methods**: We utilized the Parkinson's Progression Markers Initiative (PPMI) database sponsored by The Michael J. Fox Foundation. This dataset provides unique access to longitudinal data from 423 newly diagnosed PD patients. Data from the Movement Disorder Society Unified PD Rating Scale (MDS-UPDRS) Part II and III from all PD patients at all time points were aggregated. To identify distinct motor subtypes without using *a priori* models, we applied correlational hierarchical clustering to this data set. We classified these groups based on the most frequent MDS-UPDRS questions that each group included. We then assigned all patients subtypes for each visit. **Results**: After removing instances with missing data, 3,650 motor-assessment time points from 423 patients were used for hierarchical clustering. The majority (78%) of time points were within 5 years of diagnosis. We found three broad groups of MDS-UPDRS questions: a TD group, an Intermediate group, and a PIGD group. The specific MDS-UPDRS questions assigned to the TD and PIGD groups align well with those set forth by Stebbins et al., 2013 for calculating a Tremor-score and PIGD-score, respectively. Additionally, three specific subgroups within the Intermediate group emerged: an axial-predominant subgroup, an appendicular-predominant subgroup, and a rigidity-predominant subgroup. Subtypes were then assigned to each time point with the following frequencies: 73% TD, 1% Axial, 4.5% Appendicular, 2.5% Rigidity, and 18% PIGD. When examining subtype changes over time, results suggest that the cohort as a whole progressed away from a TD phenotype and towards a PIGD subtype with disease progression, $F(1,8) = 24.51$, $p = 0.001$. **Conclusions**: Using MDS-UPDRS assessment data, we identified at least 5 distinct motor subtypes of early PD: TD, Axial, Appendicular, Rigidity, and PIGD. Consistent with the literature, the majority of early PD patients are classified as TD. Motor subtype designation may be influenced by disease duration.

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5-HT$_3$ Receptor inhibition in spinal cord mediates Visceral Hypersensitivity in Serotonin Transporter Knockout (SERT KO) Rats

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The Irritable bowel syndrome (IBS) is a chronic functional gastrointestinal disorder characterized by altered bowel habits and visceral hypersensitivity, especially in women. 5-Hydroxytryptamine (5-HT, serotonin) signaling is disrupted in some IBS patients possibly due to polymorphic variations in the gene encoding the serotonin transporter (SERT) which result in increased extracellular 5-HT availability. Female SERT knockout (KO) rats exhibit visceral hypersensitivity to colonic distention that mimics colonic hypersensitivity known to occur in female IBS patients. Alosetron, a 5-HT$_3$ receptor antagonist is used to treat symptoms in female IBS-diarrhea patients. The mechanism of action of alosetron is not fully understood because it crosses the blood brain barrier (BBB). Therefore the analgesic effects of 5-HT$_3$ receptors maybe mediated peripherally and/or centrally. **Aims.** We tested the hypothesis that 5-HT action at central 5-HT$_3$ receptors contributes to visceral hypersensitivity. **Methods.** The visceromotor response (VMR) to colorectal distension (CRD) was used to assess visceral hypersensitivity in SERT KO and wild type (WT) female rats. We tested subcutaneous (s.c.) and intrathecal (i.t.) administration of morphine and the BBB-permeable 5-HT$_3$ receptor antagonists alosetron

and granisetron and the partially BBB-permeable 5-HT3 receptor antagonist ramosetron (s.c.). **Results**. Ramosetron (0.1 mg/kg, s.c., a peripherally selective dose) did not affect visceral sensitivity, while alosetron (0.1 mg/kg, s.c.), granisetron (0.1 mg/kg, s.c.), and ramosetron (1 mg/kg, s.c., a dose that would inhibit central 5-HT3 receptors) increased visceral hypersensitivity in SERT KO female rats. Granisetron (25 nmol, i.t.) did not affect visceral sensitivity, while alosetron (25 nmol. i.t.) increased visceral sensitivity. Morphine (3 mk/kg, s.c. or 10 µg, i.t.) suppressed visceral hypersensitivity in all rats. **Conclusion**. The increase in visceral hypersensitivity in SERT KO female rats treated with 5-HT receptor antagonists is mediated by blockade of spinal 5-HT3 receptors. Constitutive KO of SERT suppresses the contributions of peripheral 5-HT3 receptors to visceral hypersensitivity and central 5-HT3 receptors become predominant. Supported by: R01DK103759.

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Cardiolipin Promotes Mitochondrial Localization of NLRP3 and Caspase-1 to Prime Inflammasome Assembly

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Pattern recognition receptors (PRR) coordinate innate immune responses by sensing molecular signals associated with infection or injury. Nucleotide-binding, leucine rich repeat, and pyrin domain-containing protein 3 (NLRP3) is a cytosolic PRR that is activated downstream of a broad range of infectious and sterile insults. NLRP3 orchestrates inflammatory responses by forming a macromolecular inflammasome complex with the adaptor molecule apoptosis associated speck-like protein containing a caspase recruitment domain (ASC) and the cysteine protease caspase-1. Inflammasome activation culminates in caspase-1 autoactivation and IL-1β and IL-18 maturation. While NLRP3 protects against bacterial, fungal, and viral pathogens, aberrant NLRP3 activation is implicated in several inflammatory diseases. NLRP3 activation is a two-step process that requires an initial nuclear factor kappa B (NF-κB)-activating priming stimulus followed by a secondary NLRP3-specific agonist. While there is enormous molecular diversity among NLRP3 agonists, this second signal appears to engage a common pathway involving cation flux. Furthermore, NLRP3 associates with mitochondria and mitochondrial damage is implicated in NLRP3 activation, although the precise role for mitochondria in NLRP3 activation is controversial. We previously demonstrated that the mitochondrial phospholipid cardiolipin binds to NLRP3 and is required for inflammasome activation. Here, using a broken cell system, we determined that liposomes containing physiologic densities of cardiolipin promote NLRP3-dependent inflammasome activation. However, we also found that caspase-1 could directly interact with cardiolipin and that high density cardiolipin liposomes induced inflammasome-independent caspase-1 oligomerization and autocatalysis. Finding that NLRP3 and caspase-1 are independently capable of binding to cardiolipin, we more closely examined the association of inflammasome components with mitochondria. In contrast to previous studies suggesting NLRP3 associates with mitochondria during activation, we found that both NLRP3 and caspase-1 independently localize to outer mitochondrial membranes (OMM) in response to the initial priming stimulus. The association of NLRP3 and caspase-1 with mitochondria was dependent on increased mitochondrial reactive oxygen species (ROS) production during priming. Mitochondrial ROS generated at priming also triggered the externalization of cardiolipin to the OMM. Movement of the adaptor ASC to the mitochondria and inflammasome activation occurred after the second activating signal and required both cytosolic calcium elevation and the preceding mitochondrial association of NLRP3. Collectively, our findings illustrate that mitochondria serve as innate immune signaling platforms through multiple stages of NLRP3 inflammasome activation. Further, paralleling lipid A interactions with caspase-11, we have demonstrated that caspase-1 is capable of binding to the phospholipid cardiolipin.

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Histone Deacetylase 3 Prepares Brown Adipose Tissue For Acute Thermogenic Challenge

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Brown adipose tissue (BAT) is a thermogenic organ that dissipates chemical energy as heat to protect animals against hypothermia and to counteract metabolic disease. However, the transcriptional mechanisms that determine BAT thermogenic capacity prior to environmental cold are unknown. We show here that Histone Deacetylase 3 (HDAC3) is required to activate BAT enhancers to ensure thermogenic aptitude. Strikingly, mice with BAT-specific genetic ablation of HDAC3 become severely hypothermic and succumb to acute cold exposure. UCP1 is nearly absent in BAT lacking HDAC3 and there is also marked down-regulation of mitochondrial oxidative phosphorylation (OXPHOS) genes. Remarkably, although HDAC3 acts canonically as a transcriptional corepressor, it functions as a coactivator of Estrogen-Related Receptor a (ERRa) in BAT. HDAC3 coactivation of ERRa is mediated by deacetylation of PGC-1a and is required for the transcription of *Ucp1*, *Pgc-1a*, and OXPHOS genes. Thus, HDAC3 uniquely primes Ucp1 and the thermogenic transcriptional program to maintain a critical capacity for thermogenesis in BAT that can be rapidly engaged upon exposure to dangerously cold temperature.

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IQGAP1-mTORC1 Interaction Coordinates Lipid Metabolism

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Dysregulation of lipid metabolism in the liver is associated with a number of diseases including obesity, insulin resistance, non-alcoholic fatty liver disease, and cancer. The mechanistic target of rapamycin (mTOR) complex 1 (mTORC1) is a kinase signaling complex that regulates fed state metabolism and has been implicated in a number of these conditions. Multiple components of mTORC1 bind to IQ motif-containing GTPase Activating Protein 1 (IQGAP1), a multifunctional scaffolding protein. However, the metabolic impact of IQGAP1 is yet to be elucidated. *The objective of this study is to identify the role for the scaffolding protein IQGAP1 in regulating lipid metabolism.* **Methods:** Adult male 129/SVJ wild-type and *Iqgap1*$^{-/-}$ mice were either fed normal chow *ad libitum*, or fasted 24 hours with

access to water, or fed a ketogenic diet (calories from fat – 90.5%, protein – 9.1%, and carbohydrates – 0.4%) for 4 weeks. Liver, gonadal white adipose tissue (gWAT), and serum were collected from these animals for analysis using a variety of techniques including qPCR, western blot, histology, and biochemical serum assays. **Results:** Hepatic IQGAP1 expression was induced by a 24 hour fast, suggesting that IQGAP1 may participate in the fasting response. However, fasting-mediated ketogenic genes and serum ketone body levels did not differ between *Iqgap1*$^{-/-}$ and WT mice. Since mTORC1 is active in the fed state, we next assessed the activation of mTORC1 in fed WT and *Iqgap1*$^{-/-}$ mice. Excitingly, phosphorylation of the bona fide mTORC1 target S6K1 was dramatically reduced in *Iqgap1*$^{-/-}$ mice, which indicates that IQGAP1 is important for mTORC1 activity. Notably, mTORC1 activation was restored by ectopic overexpression of IQGAP1 in the livers of *Iqgap1*$^{-/-}$ mice. mTORC1 regulates fatty acid synthesis by increasing the activity of the nuclear receptor SREBP1c. In line with the decreased mTORC1 activity, hepatic gene expression of *Srebp1c* and its target *Fasn* were decreased in *Iqgap1*$^{-/-}$ mice. Furthermore, *Iqgap1*$^{-/-}$ mice have lower serum triglycerides and 20% smaller gWAT depots. This phenotype is exacerbated under ketogenic diet conditions, where *Iqgap1*$^{-/-}$ mice accumulate 30% less gWAT compared to the WT animals. Interestingly, ketogenic diet resulted in higher hepatic triglyceride content but reduced levels of serum ketone bodies in *Iqgap1*$^{-/-}$ mice, which reflect improper lipid storage along with a defective ketogenesis in the liver. It is known that elevated mTORC1 activation inhibits ketogenesis, so we examined the level of mTORC1 activity under ketogenic conditions and found it elevated in *Iqgap1*$^{-/-}$ mice compared to WT. This result is in contrary to the reduced mTORC1 levels observed in the fed state in *Iqgap1*$^{-/-}$ mice suggesting that the nutrient state drives the IQGAP1-mTORC1 interactions. **Conclusions:** Scaffolding protein IQGAP1 is required for proper regulation of lipid metabolism by mTORC1 under both fed and ketogenic nutritional states.

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Myristoylated Alanine-Rich C-Kinase Substrate Phosphorylation Enhances the Growth and Radiation Resistance of Glioblastoma

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Glioblastoma multiforme (GBM) is the most common and deadly form of glioma, with a median survival of 14 months. GBM remains a deadly disease due to its diffuse nature, high proliferative capacity and therapeutic resistance. Improving our understanding of these tumor promoting properties is vital to improving therapeutic responses. Myristoylated Alanine-Rich C-Kinase Substrate (MARCKS) is a natively unstructured protein found in GBM that can influence tumor growth and radiation resistance through its ability to electrostatically sequester the signaling phospholipid phosphatidylinositol (4,5)-bisphosphate (PIP2), vital for intracellular calcium signaling and AKT activation, while unphosphorylated. AKT activation is associated with tumor growth and radiation resistance. 92% of GBM's have mutations in the pathways that enhance the production of these tumor promoting phospholipids making MARCKS significant in its ability to suppress multiple common mutations through sequestration of the phospholipid substrate. MARCKS remains poorly understood in cancer with overexpression having opposite effects across cancer types when its phosphorylation status is ignored. Understanding the impact of MARCKS phosphorylation in GBM will reveal its role in tumor progression as-well-as its potential as a therapeutic target. **Methods** Doxycycline inducible MARCKS mutants were generated in U87 GBM cells to test the effects of MARCKS phosphorylation on tumor growth and radiation sensitivity. Growth was assessed in-vitro by colony formation assay and ATPlite. Radiation sensitivity was assessed in-vitro using clonogenic assay and γH2AX foci formation. Impact of MARCKS phosphorylation on overall survival and radiation sensitivity was assessed in-vivo via intracranial injection into athymic nude mice receiving doxycycline chow. **Results** Overexpression of MARCKS (WT+) and a non-phosphorylatable (NP) variant led to a decrease in colony number and size compared to a control vector in the colony formation assay, while the pseudo-phosphorylated (PP) MARCKS mutant had a highly significant (P **Conclusions** The phosphorylation status of MARCKS dictates whether MARCKS functions as a tumor suppressor and radiation sensitizer or an enhancer of tumor growth and survival, and may be an ideal therapeutic target.

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Inhalation of Gas Metal Arc-Stainless Steel Welding Fume Promotes Lung Tumorigenesis in A/J Mice

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Epidemiologic studies suggest an increased risk of lung cancer with exposure to welding fumes, but controlled animal studies are needed to support this association. Oropharyngeal aspiration of gas metal arc-stainless steel (GMA-SS) welding fume has been shown by our laboratory to promote lung tumor formation in vivo using a two-stage initiation-promotion model. Our objective in this study was to determine if GMA-SS fume also acts as a lung tumor promoter when delivered via inhalation to lung tumor susceptible mice. Male A/J mice received intraperitoneal (IP) injections of corn oil or the chemical initiator 3-methylcholanthrene (MCA;10 μg/g) and one week later were exposed by whole body inhalation to air or GMA-SS welding aerosols for 4 h/d x 4 d/w x 9 w at a target concentration of 40 mg/m^3. Lung nodules were enumerated at 30 weeks post-initiation. GMA-SS fume significantly promoted lung tumor multiplicity in A/J mice initiated with MCA (16.11 ± 1.18) compared to MCA/air-exposed mice (7.93 ± 0.82). Histopathological analysis found that the increased number of lung nodules in the MCA/GMA-SS group were hyperplasias and adenomas, which was consistent with developing lung tumorigenesis. Lung metal deposition analysis revealed that a markedly lower deposited dose (approximately 5 fold) elicited a similar fold-change lung tumorigenic response when compared to our previous aspiration study. In conclusion, this study demonstrates that inhalation of GMA-SS welding fume promotes lung tumor formation in vivo and provides further support for the epidemiologic studies that show welders are at an increased risk for lung cancer.

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Risk Factors for Anal Dysplasia in HIV+ Individuals

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The prevalence of anal cancer in HIV+ individuals has been increasing steadily. Such individuals are also at an increased risk for its precursor lesion, anal intraepithelial neoplasia (AIN) with rates up to 36% for men and 26% for women. More than 90% of anal cancers can be attributed to a continual presence of human papillomavirus (HPV), which is also quite common in HIV+ persons with rates from 50-90%. The current methods used to prevent anal cancer are annual Pap smears, biopsies, and precancerous lesion treatment. There is a necessity for the development of biomarkers that will delineate those who need intervention. The rate of cervical dysplasia in women increases by 30% with the co-shedding of Epstein Barr Virus (EBV) and HPV as opposed to just high-risk HPV. Due to the common epidemiology and histopathology of anal and cervical cancer, EBV could also be a biomarker for anal dysplasia. The goal of this study is to identify the risk factors, including EBV anal shedding, for anal disease in HIV+ individuals. **Methods:** Informed consent was taken from stable HIV+ patients who were attending HIV care clinics at University Medical Center and Ochnser Medical Center. A questionnaire discussing demographics and behavior was administered. Anal swabs were collected for Pap smear analysis and HPV and EBV virus detection by reverse line blot and conventional PCR respectively. High resolution anoscopy was performed on those with abnormal anal cytology and biopsies taken when clinically indicated. Peripheral HIV viral load and CD4 count within 3 months of the visit were obtained. Risk factors for anal dysplasia were assessed using SPSS software. **Results:** The population (n=188) was 88.3% male, 54.3% African American, with a mean age of 49.1, mean CD4 count of 523 cells/ml, and median HIV viral load of 39 copies/ml. High-risk HPV was detected in 67.9% and EBV in 28.8% of the anal specimens, and co-detection in 23.2%. Anal dysplasia was seen in 29.8% (3.2% HSIL) and abnormal anal Pap in 59.1%. Interestingly, 50% of women in this study (n=11) were positive for anal dysplasia. Men who have sex with men (MSM) were more likely to have anal dysplasia, at approximately 33.7% compared with non MSM (15.8%) with p=.098. The most notable risk factors for anal dysplasia were individuals with CD4 cell counts less than 200 (50.0% vs. 29.7%, p=.060) and co-detection of High Risk-HPV and EBV (47.1% vs. 27.5, p=.029). **Conclusions:** High rates of anal dysplasia (29.8%) were found in many HIV+ individuals. Those with CD4 cell counts lower than 200 and those who had both high risk HPV and EBV were at higher risk of anal dysplasia. As well, a high percentage of MSM were found to have anal dysplasia. Focused screening of the HIV+ MSM patient with lower CD4 cell counts is warranted, as are strategies that can stratify patients based on their anal HPV/EBV status.

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Transcription Factor 21 Mediates Congenital Anomalies of the Kidney and Urinary Tracts and Controls Branching Morphogenesis in the Developing Kidney

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congenital anomalies of the kidney and urinary tract (CAKUT) are the leading cause of chronic kidney disease in children. CAKUT includes a broad variety of malformations and is considered genetically heterogeneous, stemming from alterations in genes critical for kidney development. Body of evidence points to the involvement of Transcription Factor 21 (TCF21) in early nephrogenesis and in the development of CAKUT in the mouse, although the mechanisms and signaling pathways through which it contributes to CAKUT remain obscure. Here we show that TCF21 is implicated in branching morphogenesis, one of the earliest events in kidney development. During this process, the ureteric bud (UB) invades the mesenteric mesenchyme (MM), and while the two structures exchange reciprocal inductive messages the UB, under tight transcriptional regulation, undergoes repetitive dichotomous bifurcations to form the collecting ducts whereas the MM undergoes mesenchymal to epithelial transition and gives rise to the nephrons. **Aims**: we set out to examine the morphology of TCF21-induced kidney dysplasia and to evaluate change in gene expression in TCF21 null mice kidneys and its contribution to CAKUT. **Methods**: we employed immunohistochemistry, in-situ hybridization, real-time PCR and explant studies on kidneys from a TCF21 knockout mouse model. **Results**: 1. Kidneys deficient of TCF21 showed delayed epithelialization of the MM and arrested UB branching with resultant renal dysplasia. 2. TCF21 deficient kidney explants demonstrated very abnormal UB branch morphology and reduced UB tip number suggesting impaired branching morphogenesis. 3. Transcript level of the GDNF-RET pathway, known to be critical in UB branching, was significantly down-regulated in TCF21-null kidneys. Specifically, GDNF (Glial cell line-derived neutrophic factor), WNT11 and RET were expressed at 16%, 29% and 52% of normal levels in TCF21 null kidneys at E18.5. When examined histologically, GDNF gene expression was markedly low in the nephrogenic zone of TCF21 null kidneys compared to controls. 4. In contrast to the decreased expression of the GDNF-RET axis, the expression of genes of the FGF and the canonical WNT signaling, WNT4, FGF10 and FGF7, was significantly up-regulated to 210%, 114%, 184% respectively in TCF21 null kidneys, implying activation of compensatory pathways that rescue branch induction. **Conclusion**: Taken together, these results suggest that TCF21 has a critical role in branching morphogenesis and the development of CAKUT. Specifically, TCF21-induced transcriptional signature involves the GDNF-RET axis. Further study is required to elucidate specific gene targets that are directly regulated by this transcription factor.

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Endocytic Vesicle Rupture by Amyloid Proteins Promotes Inclusion Formation through Disruption of the Autophagy-lysosome Degradation Pathway

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Numerous pathological amyloid proteins spread from cell to cell during neurodegenerative disease, facilitating the propagation of cellular pathology and disease progression. Understanding the mechanism by which disease-associated amyloid protein assemblies enter target cells and induce cellular dysfunction is therefore key to understanding the progressive nature of such neurodegenerative diseases. In this study, we utilized an imaging-based assay to monitor the ability of disease-associated amyloid assemblies to induce the rupture of intracellular vesicles following endocytosis, as well as to elucidate the cellular consequences of this damaging mechanism of invasion. We observe that the ability to induce vesicle rupture is a conserved feature of fibrillar amyloid assemblies of α-synuclein (α-syn), tau, and huntingtin Exon1 with pathologic polyglutamine repeats. Immunostaining analysis revealed that vesicles ruptured by α-syn are lysosomes, but the observation that many ruptured vesicles are positive for the autophagic marker LC3 and re-establish a low intravesicular pH suggests that ruptured lysosomes become targeted to the autophagic degradation pathway. We further note a pathological enlargement of low pH compartments containing α-syn and damaged vesicular debris, which may be due to a loss of proteolytic enzymes following lysosomal rupture and a resulting inability of autophagy to degrade the cellular burden of misfolded proteins and damaged vesicles. We observe that vesicles ruptured by α-syn can accumulate and fuse into large, intracellular structures resembling Lewy bodies in vitro, and show that the same markers of vesicle rupture surround Lewy bodies in brain sections from PD patients. These data underscore the importance of this conserved endocytic vesicle rupture event as a damaging mechanism of cellular invasion by amyloid assemblies of multiple neurodegenerative disease-associated proteins, and suggest that proteinaceous inclusions such as Lewy bodies form as a consequence of continued fusion of autophagic vesicles in cells unable to degrade ruptured vesicles and their amyloid contents.

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CDK 7/9 Inhibition Amplifies Mithramycin's Suppression of Ewing Sarcoma Cell Proliferation

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Ewing sarcoma is driven by the fusion of the EWSR1 and FLI1 genes generated by a t(11:22) chromosomal translocation. Ewing sarcoma cells depend on the EWS-FLI1 fusion product, a constitutively active oncogenic transcription factor, and become nonviable when EWS-FLI1 driven transcription is inhibited. Mithramycin, a natural antineoplastic compound, has been identified as a potent EWS-FLI1 inhibitor and is highly toxic to Ewing sarcoma cells. However, clinical trials of mithramycin in patients with Ewing sarcoma have been limited due to toxicity. To overcome this challenge, we sought to identify genes that sensitize Ewing sarcoma cells to mithramycin when inhibited. We identified RNA pol II inhibition as a potent sensitizer to mithramycin and phenocopied this with the dual cyclin dependent kinase (CDK) 7/9 inhibitors PHA-767491 and SNS-032. We show a marked suppression of cellular proliferation in response to mithramycin in combination with either CDK 7/9 inhibitor at concentrations significantly lower than mithramycin alone. **Methods:** We utilized matrix drug screening with small molecule CDK inhibitors in combination with mithramycin. We measured cell viability using MTS assays and time lapse microscopy in Ewing sarcoma cells. Results were correlated with EWS-FLI1 activity using western blot analyses and qPCR. Synergy between small molecule inhibitors and mithramycin was measured using Bliss independence. **Results:** PHA-767491 and SNS-032 displayed strong synergy when combined with mithramycin. These combinations decreased the concentration of mithramycin necessary to block Ewing sarcoma cell proliferation, measured by multiple complementary assays. These results reflected effects on EWS-FLI1 activity. **Conclusions:** We have identified a synergistic relationship between the small molecules PHA-767491 and SNS-032 with mithramycin in Ewing sarcoma cells. Future work will include siRNA validation of the CDK 7/9 inhibition and mithramycin combination, analysis of genome wide expression changes, and characterization of the efficacy of a PHA-767491 or SNS-032 and mithramycin drug combination *in vivo* with the eventual goal of introducing it into the clinic.

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Molecular Mechanisms for Behavioral Modulation by Dopamine

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Dopamine (DA) is required for voluntary movement, reward signaling, and attention control among other functions. The effects of DA on target cells have been extensively studied. By contrast, mechanisms that regulate the activity and development of DA neurons themselves are not well understood, and could be important targets for the treatment of neurological and psychiatric disorders that are associated with the dysfunction of brain DA systems. To identify novel genes that function in DA neurons, we studied the simple nervous system of the nematode *C. elegans*: they contain eight DA neurons that mediate stereotyped locomotory behaviors and are accessible to molecular, genetic and physiological analysis. We have sequenced the mRNAs of DA neurons and identified four genes whose transcripts are: (1) highly enriched in DA neurons compared to other neurons, (2) conserved with mammalian genes, and (3) produce a defect in DA-dependent modulation of locomotion upon mutating. These candidate genes are similar to mammalian genes that play important roles in excitatory neurotransmission, cell-cell adhesion, axon guidance, and cytoskeletal organization. Their precise roles in regulating DA neuron development and function remain very poorly understood. We will determine the function of these conserved proteins in DA signaling, which will advance our understanding of the mechanisms required in vivo for dopamine signaling, and of the roles this diverse group of proteins play in the nervous system.

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Impaired Natriuretic and Sympathoinhibitory Responses to Alterations in Fluid and Electrolyte Balance Accompany Age-Dependent Hypertension in Sprague-Dawley Rats

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The prevalence of hypertension correlates with increased age and elevated sympathetic tone in human subjects. We hypothesized that sodium retention and sympathoexcitation contribute to age-dependent hypertension in male Sprague-Dawley (SD) rats. **Methods:** Two-month, 8-month, and 15-month old male SD rats underwent an acute intravenous (IV) volume expansion (VE; 5% body weight) and mean arterial pressure (MAP), natriuresis (UNaV), and paraventricular nucleus (PVN) parvocellular neuronal activation (c-Fos expression) were assessed. Separate 2-month, 8-month, and 15-month old male SD rats were maintained on a normal salt (NS, 0.6% NaCl) or high salt diet (HS, 4% NaCl). On day 21, MAP, NCC activity (natriuresis to hydrochlorothiazide, HCTZ, 2mg/kg), and measures of sympathetic tone (peak depressor response to hexamethonium (30mg/kg), and plasma and renal norepinephrine (NE) levels) were assessed (n=4/group). **Results:** VE-evoked natriuresis and PVN parvocellular neuronal activation were impaired in aged rats (% sodium load excreted; 2-month 78±6 vs 8-month 60±7 vs 15-month 22±9, $P < 0.05$: PVN neuronal activation [c-fos+ cells]; medial parvocellular 2-month 59±4 vs 8-month 42±7 vs 15-month 13±5, $P < 0.05$). Aged rats on a NS diet exhibited increased MAP and NCC activity (MAP [mmHg]; 2-month 124±2 vs 8-month 140±1 vs 15-month 149±3, $P < 0.05$: peak ΔUNaV to HCTZ [µeq/min]; 2-month NS 9±1 vs 8-month NS 18±2 vs 15-month NS 17±3, $P < 0.05$). Sympathetic tone also increased with age in rats on a NS diet (plasma NE [nmol/L]; 2-month 44±4 vs 8-month 55±3, $P < 0.05$: renal NE [pg/mg]; 2-month 612±36 vs 8-month 835±48 vs 15-month 974±39, $P < 0.05$: depressor response to hexamethonium [mmHg]; 2-month -33±4 vs 8-month -64±5 vs 15-month -60±3, $P < 0.05$). Chronic HS-evoked suppression of NCC activity and plasma NE was attenuated in 8-month old rats (peak ΔUNaV to HCTZ [µeq/min]; 2-month NS 9±1 vs HS 7±1, $P < 0.05$: 8-month NS 18±2 vs HS 16±1, ns: plasma NE [nmol/L]; 2-month NS 44±4 vs HS 28±4, $P < 0.05$: 8-month NS 55±3 vs HS 42±4, $P < 0.05$). **Conclusion:** Age-dependent hypertension is accompanied by impaired natriuretic and sympathoinhibitory responses to acute and chronic challenges to fluid and electrolyte homeostasis. We speculate that in aged animals, elevated sympathetic tone-facilitated by reduced activation of PVN sympathoinhibitory neurons-increases NE-driven NCC activity, promoting sodium reabsorption and hypertension. These findings suggest that therapies reducing sympathetic outflow and sodium retention may be particularly useful in older hypertensive patients given 1) evidence from the PATHWAY-2 trial indicating a primary role of sodium retention in treatment-resistant hypertension and 2) excessive dietary sodium intake in the global population.

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Neuroimmune Mechanisms Inhibit Cognitive Recovery from Viral Encephalitis

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Altered neuroimmune communication contributes to neurologic dysfunction in a variety of disorders of divers etiology including those of autoimmune, neurodegenerative, and neuroinfectious origin. In viral encephalitis, immune cell infiltration into the central nervous system (CNS) is critical for effective pathogen clearance; however little is known about how inflammatory mechanisms necessary for survival of the organism may interfere with normal cognitive function. Using a murine recovery model of West Nilve Virus encephalitis, we demonstrate that activated microglial and memory T cells persist in the CNS for weeks after viral clearance and contribute to spatial learning deficits. Animals deficient in interferon gamma signaling, an important T-cell derived antiviral cytokine, demonstrate a worsened clinical course and delayed viral clearance. However, despite experiencing more severe virologic disease, these animals showed complete protection from spatial learning deficits. These results suggest that post-infectious alterations in neuroimmune signaling may contribute to neurologic sequelae, providing a novel therapeutic target to combat neurologic dysfunction of viral origin.

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The Biomechanical Effects of Lower Extremity Instability on Segmental Sequencing Amongst Female Handball Athletes

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Throwing is a kinetic chain activity requiring coordinated energy transfer from the lower extremity (LE), through the trunk, to the upper extremity (UE), and through the hand for ball release. Literature has shown inadequate strength throughout the body may contribute to inefficient force production and decrease optimal energy transfer for throwing performance. The sport of team handball (THB) is unique in that it has both side-to-side cutting and overhand throwing. THB requires dynamic movements, involving segmental stability throughout the kinetic chain. The ability to transfer energy and perform an accurate shot on goal is dependent on the synchronization and strength of the UE and LE. The lumbopelvic-hip complex (LPHC) is a key component in connecting these two components. Previous research has shown that proper stabilization of the LPHC leads to higher rotational velocities across all segments. Interruptions in this energy transfer may lead to decreased performance and injury susceptibility. In THB, shoulder and knee injuries account for 44% and 26.7% of all injuries, respectively. Even though the injury rates and the importance of energy transfer throughout the kinetic chain are known, there has yet to be a comparison examining LE segments and their contribution to the UE segments in THB athletes. Therefore, the purpose of this study was to examine the relationship between LE stability and segmental sequencing during a jump shot. It was hypothesized that LE instability would affect segmental sequencing of the pelvis (PV), torso (TV), humeral (HV), and forearm velocities (FV). **Methods:** Twenty female THB athletes (26.5±4.7 years; 174.6±4.2 cm; 74.4±6.4 kg) participated. An electromagnetic tracking system was

used to collect kinematic data (100Hz) while participants performed three 9-meter jump shots. Data were analyzed across six events: foot contact (FC), toe off (TO), maximum shoulder external rotation (MER), ball release (BR), maximum shoulder internal rotation (MIR), and landing (LA). Knee valgus at LA was used to classify participants' LE stability. Knee valgus greater than 17° is considered unstable. **Results:** Mann-Whitney U-test was used for analysis. Statistically significant differences were found in the following groups ($p \leq 0.046$): PV and TV between groups at all events; HV at FC, MER, and BR; and FV at events FC, MER, BR, and MIR. **Discussion:** These findings may suggest the difference in throwing mechanics could be affected by LE instability for this select group of female THB athletes. These differences could infer increased risk of injury in the UE and LE when landing from a jump shot. Since the LPHC plays a major role in the transfer of energy from the LE to the UE, a focus of stabilization in this area could benefit these athletes. It is recommended that further investigations also consider muscle activation throughout the throwing motion.

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Novel Super-enhancer Screen Identifies Cancer Stem Cell Dependencies in Glioma

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Glioblastoma is the most prevalent and aggressive primary brain tumor in adults and is among the most lethal of all human cancers with poor responses to all therapeutic modalities. Functionally defined glioma stem cells (GSCs) are proposed to drive these poor clinical outcomes by mediating therapy resistance, self-renewal, and maintenance of cellular heterogeneity. **Methods**: To understand the cell-type-specific molecular processes that underlie GSC maintenance and tumorigenicity, I profiled super-enhancers in GSCs through analysis of active enhancer histone modification data (H3K27ac) in matched GSC and differentiated glioma cell (DGC) samples. Super-enhancers, defined as clusters of typical enhancers enriched for the binding of transcription factors, function as master epigenetic regulators that define cell state, regulate genes controlling cell identity, orchestrate cell-type dependent transcriptional networks, and are found at key oncogenic drivers in a variety of cancers. **Results**: To identify genes important in defining GSC identity, I identified gained super-enhancers unique to glioma stem cell populations and linked each super-enhancer to the closest differentially expressed gene based on matched RNA-seq data. Through interrogating gene expression and survival data from The Cancer Genome Atlas (TCGA), I chose to further investigate only those genes for which high expression was associated with poor patient prognosis. Through this approach, I identified lipid biosynthesis as a potential epigenetically regulated GSC dependency pathway. I found that lipid metabolism gene expression is increased in GSCs compared to matched DGC controls and that knockdown by shRNA is associated with decreased cell growth. In a validation cohort, I showed that GSC lines containing the identified super-enhancer exhibit high expression of a lipid metabolism gene, suggesting that the expression of this gene is epigenetically driven. Next, I analyzed the upstream transcription factor control circuits that underlie a critical super-enhancer in this pathway. I found that expression of two neurodevelopmental transcription factors, OLIG2 and SOX2, is significantly positively correlated with that of lipid metabolism genes in clinical samples and that occupancy of these factors may drive the persistence of this super-enhancer. **Conclusions**: Super-enhancer screens serve as a useful mechanism for the identification of cell-type specific biology. I utilized epigenetic markers of cell-type specific functional significance to identify a potential pathway of oncogenic metabolic reprogramming in GSCs and demonstrated that lipid biosynthesis is selectively upregulated in GSCs, is critical for cell growth, and may be driven through an epigenetic mechanism. This discovery implicates aberrant lipid metabolism networks in glioma pathogenesis and suggests that targeting of fatty acid synthesis may provide a specific way to more effectively treat patients with glioblastoma.

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Fast-acting, Heat-stable Single Chain Insulin Therapeutics Promise Enhanced Global Access to Insulin Replacement for the Treatment of Diabetes Mellitus

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Single-chain insulin analogs (SCIs) are degradation-resistant therapeutics that show promise to enhance access to insulin-based glycemic control in technology-limited areas where refrigeration is scarce. Due to the increased risk of insulin overdose in these regions, fast-on/fast-off blood glucose reduction is highly desired to avoid prolonged hypoglycemic episodes and subsequent morbidity and mortality. While fast-acting clinical therapeutics (e.g., insulin *lispro*) are widely used in developed nations, these two-chain insulin formulations have extremely limited hot storage shelf lives, making them difficult to effectively utilize in areas that experience high year-round temperatures. **Approach:** Using structure-based design principles, we demonstrate a novel set of fast-acting, heat-stable single chain insulin analogs. Each SCI consists of a six residue peptide linker [EEGPRR] that confers marked resistance to heat-induced degradation and also enables straightforward and large scale synthesis of natively folded insulin when expressed in *Pichia pastoris* yeast. Based on prior studies in two-chain molecules, single-chain positions A8 and A14 were mutated relative to the wild-type Tyr^{A14} and Thr^{A8} to determine both their isolated and integrated impact on protein stability as well as hormone function in diabetic Sprague-Dawley rodents. **Results:** Replacement of wild-type Tyr^{A14} with Glu^{A14} results in a dramatic 1kcal/mol increase in thermodynamic stability; replacement of wild-type Thr^{A8} with His^{A8} enhances stability by an additional 0.5kcal/mol. Whereas two-chain control insulin *lispro* degrades quickly and irreversibly when heated above 40°C, all tested SCI analogs remain undamaged when heated up to 88°C. Following subcutaneous injection in diabetic rodents, SCIs containing Thr^{A8} are fast-acting with pharmacodynamics profiles highly similar to that of insulin *lispro* whereas His^{A8} induces markedly delayed blood glucose recovery (i.e., long-acting). Additionally, Tyr^{A14}-containing analogs appear to drop blood glucose faster than Glu^{A14} analogs, which may reflect altered insulin oligomer dissociation rates and thus subcutaneous absorption. **Conclusion:** While variation of position A8 is known to alter stability and hormone potency in two-chain insulin, it appears to have an unexpectedly strong effect on SCI duration-of-action *in vivo*. This suggests that well-established physicochemical

determinants of protein stability and function in two-chain insulin behave differently in the context of a therapeutic single-chain insulin scaffold. We have demonstrated two novel SCI analogs (Thr^{A8}+Glu^{A14} and Thr^{A8}+Tyr^{A14}) that are as fast acting as two-chain insulin *lispro* yet considerably more heat-stable and resistant to degradation. Consequently, this work has the potential to improve the clinical care of hundreds of millions of diabetes patients worldwide.

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Phenyl-amino-methyl-quinolinols are a New Class of Antimicrobial Compounds that Potently Target the cAMP Pathway in *Plasmodium Falciparum*

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In vitro evolution and whole-genome sequencing are increasingly used to discover the mechanism of antimicrobial compounds found in high-throughput phenotypic screens, such as those that have been used to identify several antimalarial drugs that are currently in clinical trials. Identifying the mechanism of antimicrobial compounds is an important step in translating these findings into clinically approved drugs. In addition, this approach can be used to identify antimicrobial drug targets for target-based drug discovery programs, which can then identify compounds with improved efficacy and safety, as well as translate drug classes across different organisms. We use directed evolution and comparative chemogenomics to show that a class of highly potent pre-clinical antimalarial compounds, the phenyl-amino-methyl-quinolinols (PAMQs), inhibit replication of *P. falciparum* through inhibition of the production of cAMP by adenylyl cyclase (AC). We show that this effect is selective for homodimeric ACs with the F1718 genotype and that PAMQs do not significantly affect cAMP levels in human cells. In addition, these compounds also show activity against other human pathogens that contain ACs with the F1718 genotype, including fungi, kinetoplastid parasites, such as *Trypanosoma* and *Leshmania*, helminthic pathogens such as *Schistosoma mansoni*, and the mycobacterium *M. tuberculosis*. These findings suggest that the cAMP pathway is a chemically validated, druggable pathway for development of new antimicrobials, and that the PAMQs should be optimized against the malaria parasite and other eukaryotic pathogens.

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Dexamethasone Therapy does not Prevent the Recruitment of Monocyte-derived Alveolar Macrophages During Lung Fibrosis

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Idiopathic Pulmonary Fibrosis (IPF) is a progressive, disabling and ultimately fatal disease for which there are no effective therapies. A large clinical trial found that the use of systemic glucocorticoid therapy to patients with IPF, the standard of care at the time, was associated with no improvement and perhaps some harm. These data have been interpreted to suggest that inflammation is dispensable for the development of fibrosis. As we and others have found that recruited monocytes play a role in the development of fibrosis in the bleomycin model, we sought to examine the effect of glucocorticoids on macrophage populations in the lung during bleomycin-induced fibrosis. **Methods.** Ten to twelve week old C57Bl/6 mice were treated with dexamethasone (0.2 mg/kg, ip, qday) beginning one day prior to the administration of bleomycin and continuing over the course of therapy. Bleomycin (0.025IU/mouse) was instilled intratracheally and the severity of lung fibrosis was measured 21 days later using quasi-static lung compliance (Flexivent), total lung collagen in lung homogenates (picrosirius red dye precipitation), examination of Trichrome stained histologic lung sections (Ashcroft scores) and serial CT imaging (days 5, 10, 15, 21). At the same time point, lungs were digested and analyzed using multi-color flow cytometry. **Results.** In response to bleomycin, all measures of lung fibrosis including total lung collagen, lung compliance, Ashcroft scores, quantification of lung fibrosis with microCT imaging and mortality were similar in the saline and dexamethasone treated mice. Consistent with the known effects of corticosteroids, all lymphocyte population and eosinophils were reduced in dexamethasone treated mice. The number of tissue resident macrophages were reduced in mice treated with dexamethasone, while the number of monocyte derived alveolar macrophages was similar. **Conclusions.** The systemic administration of dexamethasone does not alter the severity of bleomycin-induced fibrosis. Dexamethasone therapy results in reduced numbers of tissue-resident macrophages, lymphocytes and eosinophils in the lung during bleomycin-induced fibrosis but does not alter the recruitment of monocyte-derived alveolar macrophages. Corticosteroids are unlikely to reduce the recruitment of profibrotic monocyte-derived alveolar macrophages to the lung during fibrosis.

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BORG: Role for a Novel lncRNA in Mediating Breast Cancer Metastasis and Dormancy

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Although greater than 90% of breast cancer (BC) related mortality can be directly attributed to metastases, the molecular mechanisms essential to the dissemination of primary BC tumor cells and their subsequent establishment of malignant lesions in distant tissues remain incompletely understood. The clinical detection and eradication of disseminated BC cells is complicated by their frequent acquisition of a dormant cellular state upon arrival to their metastatic niche, followed by their subsequent reactivation of proliferative programs that permit the generation of overt metastatic recurrences years-to-decades after the initial implementation of treatments targeting the primary tumor. Through *in silico* and biological analyses, we have identified a novel long noncoding RNA, BMP/OP-Responsive Gene (BORG), whose expression directly correlates with aggressive BC phenotypes and metastatic competence, including susceptibility to metastatic relapse, in multiple clinical cohorts. Within the established D2.HAN model of BC dormancy, BORG expression levels are significantly diminished in dormant D2.OR cells as compared to their metastatic D2.A1 counterparts. Importantly, shRNA-mediated knockdown of BORG expression in D2.A1 cells deters their metastatic outgrowth both *in vitro* and *in vivo*, while enforced expression of BORG in D2.OR cells confers metastatic features to them both *in vitro* and *in vivo*. Mass spectrometry analysis of captured proteins co-immunoprecipitated with BORG reveals multiple BORG-interacting proteins, including the E3 SUMO ligase TRIM28, a transcriptional co-repressor and scaffolding protein for histone and DNA modifying

enzymes that has been shown to enhance BC cell proliferation. CRISPR/Cas9-mediated knockout of TRIM28, as well as expression of mutant BORG transcripts deficient in TRIM28 binding, both prevent the BORG-mediated metastatic outgrowth of D2.OR cells. Mechanistically, chromatin immunoprecipitation assays reveal that BORG enhances the binding of TRIM28 at multiple genomic loci with which it is known to associate. This increased localization enhances TRIM28-mediated pausing of RNA Polymerase II at the promoters of these loci, which thereby inhibits transcription of these genes, including two known tumor suppressor genes, *p21* and *gadd45a.* Correspondingly, genome-wide transcriptional analyses of BORG-expressing D2.OR cells grown in 3D organotypic cultures reveal a TRIM28-dependent transcriptional signature harboring broad amplification of cellular proliferation and survival pathways. These findings imply that BORG acts as a novel driver of the genetic and epigenetic alterations operant during the acquisition of metastatic traits in BC progression, namely in the ability of disseminated cells to escape from metastatic dormancy.

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Quantifying the Posttranscriptional Effects of Thousands of Common Genetic Polymorphisms In Tandem

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Rapid advances in human genome sequencing technologies over the past decade have unraveled innumerous loci containing variants that cause inherited disease or have been placed under positive selective pressure to protect against acquired diseases such as infectious agents, climate change, and diet restrictions. However, it is still difficult to isolate the "causal" variants within each of these loci from the hundreds of neutral variants to which their genotypes are closely linked. We have developed an experimental tool to individually screen thousands of common genetic variants for posttranscriptional regulatory function. This has allowed us to pinpoint a small set of candidate variants with quantifiable posttranscriptional regulatory effects that may translate to adaptive phenotypes, and has revealed global insights into the impact of common polymorphisms at multiple stages of gene regulation.

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Are there Gender and Racial/ethnic Differences in Problem Behavior in Children 9 years of age?

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Child problem behavior negatively impacts children's adult quality of life. Although behavior may vary by gender and race/ethnicity, examinations of these differences with regard to specific types of behavior problems are limited. Our purpose is to describe differences in internalizing and externalizing problem behavior by gender and race/ethnicity in children 9 years of age. **Methods**: We use data from the Fragile Families and Child Well-Being Study (N=3,337). The Child Behavior Checklist was used to assess behavior, where higher scores indicate greater behavioral problems. We use analysis of variance (ANOVA) methods to compare behavior across gender and race/ethnicity. Unadjusted ordinary least squared regression models are used to model problem behavior on gender and race/ethnicity. Adjusted models control for maternal, family, and household variables. Behavior scores are standardized to a mean of 0 and standard deviation of 1. Statistical significance was set at the 0.05 level. **Results**: ANOVA methods indicated that internalizing behaviors were not significantly different across gender. However, male children had an average externalizing behavior score (range 0-68) of 4.55, compared to a score of 3.66 among females ($p < 0.0001$). Internalizing ($p=0.0002$) and externalizing ($p=0.0001$) scores significantly differed across race/ethnicity. In adjusted regression analyses, female externalizing behavior scores were 0.21 standard deviations lower than male scores ($p < 0.0001$). Black race was associated with lower internalizing scores by 0.20 standard deviations ($p < 0.0001$) and lower externalizing scores by 0.11 standard deviations ($p=0.028$) compared to white race. Hispanic ethnicity was associated with internalizing behaviors scores 0.27 standard deviations lower than white race ($p < 0.0001$). **Conclusion**: Although differences were relatively small in magnitude, our results highlight the need for targeted approaches for children who may receive greater benefit from interventions. Additional research is needed to understand manifestations of these differences in adolescence and adulthood.

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Allele-specific Targeting of Mutant NPM1 Induces NPM1 Nuclear Relocalization, HOX Gene Downregulation and Terminal Differentiation

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NPM1 mutated acute myeloid leukemia (AML) is a distinct entity in the WHO classification of hematopoietic cancers. It displays a specific phenotype characterized by favorable prognosis and upregulation of *HOX* cluster genes. NPM1 is a multifunctional nucleolar chaperone. Mutations in NPM1 result in cytoplasmic protein localization (NPM1c+) through the acquisition of a nuclear export signal (NES) at the C-terminus. The most frequent NPM1 mutation is a heterozygous 4bp insertion in exon 12 (mutA). The role of NPM1 in leukemogenesis is still a matter of debate and there is no direct proof that cytoplasmic localization of mutant NPM1 is necessary for maintenance of leukemia. We recently developed a CRISPR strategy for highly efficient gene editing in hematopoietic cells. We hypothesized that NPM1 mutA could be specifically targeted due to its 4bp insertion, avoiding the WT allele. Using an sgRNA spanning the insertion site (NPM1c sgRNA), we aimed to introduce indels adjacent to the mutation and disrupt the C-terminal NES. To test our hypothesis, we used the *NPM1* mutated OCI-AML3 cell line. After transfection of OCI-AML3 with NPM1c sgRNA, while *NPM1*mutA allele showed 70-90% indel frequencies, the *NPM1*wt allele was intact. To determine whether the novel edited alleles generated NPM1 that was re-localized to the nucleus, we cloned the alleles into a *GFP-NPM1* fusion construct and observed nuclear localization after transfection. Consistent with this finding, immunofluorescence revealed that edited OCI-AML3 cells had lost nearly all cytoplasmic NPM1. Return of NPM1 protein to the nucleus was followed by terminal differentiation and cell cycle arrest in G1 phase (controls 45±3%, NPM1c sgRNA 68±1.5%). Cell growth (3.7-4 fold decrease in cell counts in NPM1c sgRNA samples), colony forming ability (16-20 fold reduction in colonies in NPM1c sgRNA samples) and engraftment in xenograft models (significant loss of indels at NPM1mutA allele

in engrafted cells) were also significantly impaired after NPM1mutA targeting. Furthermore, RNA sequencing on NPM1mutA-targeted and control OCI-AML3 cells revealed almost complete loss of expression of the *HOXA* and *HOXB* cluster genes as well as *MEIS1* in treated cells (4.5 average fold reduction of *HOXA9-A13*; 5.6 average fold reduction of *HOXB7-B13*; 5 fold reduction of *MEIS1*). Allele-specific editing is a powerful tool to probe mechanistic aspects of oncogene dependencies. By achieving nuclear re-localization of mutant NPM1, we demonstrated that cytoplasmic localization of NPM1c+ is necessary for OCI-AML3 cells to maintain their leukemic phenotype. Drugs promoting mutant NPM1 nuclear localization such as CRM1 inhibitors are attractive candidates for clinical success in NPM1-mutated AML.

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Comprehensive Population-based Genome Sequencing Provides Insight into Hematopoietic Regulatory Mechanisms

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Genetic variants affecting hematopoiesis can influence commonly measured blood cell traits. To identify factors that affect hematopoiesis, we performed association studies for blood cell traits in the population-based Estonian Biobank using high coverage whole genome sequencing (WGS) in 2,284 samples and SNP genotyping in an additional 14,904 samples. Using up to 7,134 samples with available phenotype data, our analyses identified 17 associations across 14 blood cell traits. Integration of WGS-based fine-mapping and complementary epigenomic data sets provided evidence for causal mechanisms at several loci, including at a novel basophil count-associated locus near the master hematopoietic transcription factor *CEBPA*. The fine-mapped variant at this basophil count association near *CEBPA* overlapped an enhancer active in common myeloid progenitors and influenced its activity. In situ perturbation of this enhancer by CRISPR/Cas9 mutagenesis in hematopoietic stem and progenitor cells demonstrated that it is necessary for and specifically regulates *CEBPA* expression during basophil differentiation. We additionally identified basophil count-associated variation at another more pleiotropic myeloid enhancer near *GATA2*, highlighting regulatory mechanisms for ordered expression of master hematopoietic regulators during lineage specification. Our study illustrates how population-based genetic studies can provide key insights into poorly understood cell differentiation processes of considerable physiologic relevance.

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Towards Photoimmunotherapy Using Cerenkov Luminescence Excitation

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Photodynamic therapy (PDT) is a powerful and versatile tool for cancer therapy. Through a combination of photosensitizers and light to generate cytotoxic free radicals, PDT utilizes a multipronged approach to destroy cancer cells, including (1) direct cell damage mediated by reactive oxygen species (ROS); (2) damage and interruption of tumor vasculature; and (3) induction of inflammation and antitumor immune response. However, most of the photosensitizers used in PDT absorb light in or near the visible range, which has limited penetration in tissue. Thus, PDT has largely been limited to the treatment of superficial disease. This project seeks to develop novel nanotherapeutic paradigms utilizing stimulated intracellular light therapy (SILT). SILT combines low-radiance light produced by emission of high-energy charged particles from radiopharmaceuticals, termed Cerenkov radiation, with photosensitive nanoparticles that generate ROS upon photoexcitation. SILT overcomes the tissue depth limitations of PDT, while conserving its multi-cell death therapeutic pathways by generating light from within cancer cells. We have previously demonstrated efficacy of this approach for treatment of *in vitro* and *in vivo* cancer models. In our current work we will examine the use of SILT for cancer treatment, with an emphasis on its ability to induce immunotherapy.

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Alterations in Intestinal Sulfur Assimilation Metabolism Regulate Iron Homeostasis

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Sulfur assimilation is the process of incorporation of inorganic sulfate from the environment into sulfur-containing amino acids and sulfate-containing metabolites, and is a feature that is evolutionarily-conserved across bacteria, yeast, plants and mammals. A critical regulator of the sulfur assimilation pathway is bisphosphate 3'-nucleotidase (Bpnt1). Mice and humans encode two nucleotidases: Golgi-resident 3'-phosphoadenosine 5'-phosphate phosphatase (gPAPP) and the cytoplasmic Bpnt1. Loss of gPAPP in mice results in chondrodysplasia, pulmonary insufficiency, and bone joint defects, which are recapitulated in human patients carrying recessive mutations. In contrast, mice deficient for Bpnt1 ($Bpnt1^{-/-}$) develop anasarca, hepatic insufficiency, impaired ribosomal biogenesis, and age-dependent alopecia, a common manifestation of anemia. Iron is a critical dietary micronutrient, and numerous disease states result from an imbalance in iron homeostasis including hereditary

hemochromatosis, neurodegenerative disease and anemia. Thus, discovery of mechanisms affecting regulation of iron are paramount to understanding the pathophysiology of broad and varied disease states. We report that loss of Bpnt1 in mice leads to iron-deficiency anemia (IDA). Independent of dietary iron content, Bpnt1$^{-/-}$ animals display significantly lower hemoglobin, smaller mean corpuscular volume, and reduced average cellular hemoglobin (Hudson, BH, Hale, AT et al, 2016 "Modulation of intestinal sulfur assimilation metabolism regulates iron homeostasis" *to be submitted*). Strikingly, using a forward genetics approach, the brachymorphic mouse (Papss2$^{bm/bm}$), harboring a hypomorphic mutation in PAPS synthase 2, the gene that controls upstream production of PAP, crossed with the Bpnt1 global knockout animal (Papss2$^{bm/bm}$ Bpnt1$^{-/-}$- DKO), specifically reduces accumulation of PAP and rescues the anemia observed in the Bpnt1-/- animal. Next, Bpnt1$^{-/-}$ mice that were supplemented with subcutaneous iron-dextran displayed normalized Hb levels, confirming that the anemia was due to a defect in iron homeostasis rather than a defect in hematopoiesis. In addition, hepcidin RNA levels in the liver were markedly reduced, consistent with an intact hepatic response to the iron deficient state. Therefore, we hypothesized that loss of Bpnt1, and accumulation of PAP, impairs the intestinal response to iron deficiency. To investigate the role of Bpnt1 in the intestine, we generated mice with intestinal-specific deletion of Bpnt1 (Bpnt1$^{-/int}$). These mice are similarly iron deficient and display age-dependent alopecia. To understand why Bpnt1$^{-/int}$ mice were unable to respond to the low iron stress, we used an unbiased RNAseq-based approach, and show that enterocytes from Bpnt1$^{-/int}$ mice are unable to regulate key iron homeostasis factors involved in dietary iron reduction, import and transport. Interestingly, the transcriptional profile of Bpnt1$^{-/int}$ enterocytes, in part, mimic that reported from mice deficient in hypoxic-induced transcription factor, HIF-2α, which similarly develop IDA. Our studies define a new genetic basis for iron-deficiency anemia, a molecular approach for rescuing the pathophysiology and an unanticipated link between nucleotide hydrolysis in the sulfur assimilation pathway and iron homeostasis.

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Integrated Analysis of the Pancreas and Islets of a 22-year-old male with 8 years of Type 1 Diabetes

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Recent observations about the pancreas from individuals with type 1 diabetes (T1D) have highlighted the heterogeneity in clinically diagnosed T1D. To better understand the molecular changes associated with T1D, we have been studying the pancreas and islets of individuals with type 1 diabetes of < 10-years duration. Here, we present the studies of the pancreas and islets from a 22-year-old male who was diagnosed with T1D at age 14 compared to normal (n=6; 10-55y of age) and other relatively recent-onset T1D (n=3; 12-22y of age, 3-7yrs duration) donors. The 22-year-old male (BMI 25.7 kg/m^2) was reportedly noncompliant (HbA1C 11.9%) with his insulin treatment (Novolog and Lantus) and died of anoxic brain injury secondary to drug intoxication. The donor had a C-peptide measurement of 0.06 ng/mL, in the range of other T1D donors (0.02-0.26 ng/mL C-peptide; n=3), and high risk HLA haplotypes DR4, DQ2 and DQ8, but all T1D-associated autoantibodies (GAD65, mIAA, ZnT8, ICA512) were negative. By islet perifusion, the donor's beta and alpha cells had reduced insulin and glucagon secretion in response to glucose, cAMP-evoked stimulation, and KCl-mediated depolarization (relative to islet hormone content) compared to controls and T1Ds. Surprisingly, the insulin content of the donor's islets (4.57 ng/IEQ) was in the range of normal donors (1.50 - 5.21 ng /IEQ; n=5) but the glucagon content (837 pg/IEQ) was greater compared to normal donors (29 - 183 pg/IEQ; n=5) and T1Ds (57 - 618 pg/IEQ; n=3). This was further supported by analysis of the native pancreas where as many as 68.6% of the islets contained abundant numbers of beta cells. Because of the unexpected islet insulin content and islet histology, we sequenced the donor DNA for variants associated with monogenic diabetes, which revealed a heterozygous intronic mutation (IVS2-7G>A) in the glucokinase (GCK) gene, previously identified in a Canadian family (Cao et al. 2002). The donor's alpha cells, similar to T1D alpha cells, had alterations in expression of transcription factors comprising the differentiated state (NKX6.1, MAFB, ARX) by immunohistochemistry and RNA-sequencing. Notably, expression of transcription factors constituting alpha and beta cell identity improved in the donor islets following a two-month engraftment in normoglycemic, immunodeficient mice. Our results describe an individual clinically classified as T1D but who lacked humoral autoimmunity and had an unexpectedly high number of beta cells and islet insulin content. However, whether the heterozygous intronic mutation in the GCK gene was responsible for the donor's hyperglycemia is uncertain. Further studies examining the mechanism of diabetes in this donor are needed to understand what processes contributed to the hyperglycemia.

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Human Pluripotent Stem Cell-Derived Vascular Progenitors of Mesothelial Origin for Tissue Engineering and Regenerative Medicine Applications

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The mesothelium, which constitutes the outermost layer of the coelomic organs including the heart, lung, liver and gut, plays a critical role in the development, homeostasis and potentially in repair of the internal organs following injury or disease. Here, we describe methods for the efficient differentiation of human pluripotent stem cells (hPSCs) into mesothelial progenitor cells (MPCs) and define their developmental potential in both *in vitro* and *in vivo* models. Differential gene expression analysis of freshly isolated murine embryonic mesothelium was used to validate the characterization of our hPSC-derived MPCs as mesothelial in origin. Clonogenic assays were used to determine the in vitro differentiation potential of hPSC-derived MPCs into fibroblast, smooth muscle and endothelial lineages and the multipotency of hPSC-derived MPCs was evaluated in vivo by assessing integration of hPSC-derived MPCs into embryonic chick hearts and mechanically-damaged neonatal mouse hearts. At the molecular level, hPSC-derived MPCs are indistinguishable from their in vivo counterparts and respond to signaling molecules that are known to impact mesothelial cell fate decisions during development as shown by their in vitro differentiation into fibroblasts, smooth muscle cells and endothelium in response to PDGF-alpha, PDGF-beta

and Vegf signaling, respectively. When transplanted onto developing chick hearts, MPCs incorporate into the host mesothelium and invade the underlying myocardium. MPCs transplanted into mechanically-damaged neonatal mouse hearts migrate into damaged tissue along with endogenous epicardium-derived cells and assemble into coronary vessels in the repair zone. In addition to the utility of these cells for modeling mesothelial development and disease, this study opens up new avenues for tissue engineering and regeneration/repair of coelomic organs.

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Krüppel-like Factor 5 Promotes Cell Proliferation in Mouse Models of Pancreatic Ductal Adenocarcinoma

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Pancreatic ductal adenocarcinoma (PDAC) comprises 90% of pancreatic cancer cases. PDAC is believed to be derived from ductal precursor lesions, and the most common subtype of which is pancreatic intraepithelial neoplasia (PanIN). A major driver for PanIN formation is constitutively active mutant KRAS, expressed nearly universally in PDACs. Pancreas-specific expression of oncogenic KRAS cause spontaneous PanIN formation in mouse models. Krüppel-like factor 5 (KLF5) is a pro-proliferative zinc finger transcription factor that has been shown to mediate oncogenic KRAS activity in intestinal tumorigenesis. Recent evidences have shown that KLF5 plays important roles in maintaining ductal phenotype of well-differentiated PDAC and in preventing Sox4-induced apoptosis upon TGF-β stimulation. However, the exact role of KLF5 in regulating proliferation in oncogenic KRAS-mediated PDAC is unknown. **Aim:** To investigate the effect of *Klf5* depletion on proliferation of mouse pancreatic cancer cell lines derived from KC mouse model (*Pdx1-Cre; LSL-Kras^{G12D}*). **Materials and Methods:** Mouse pancreatic cell line derived from KC mouse model was treated with pharmacological inhibitors of pathways downstream of KRAS (U0126, PD98059, LY294002, SB203580, and SP600125). Changes in KLF5 protein level were examined using Western blot. Stable KC cell lines that express *Klf5*-specific shRNA (or proper scrambled shRNA control) upon doxycycline induction were established. KC cell line with *Klf5* knockout was also established using CRISPR/Cas9 system. Changes in cell proliferation were measured using cell counting and MTS assay. Cell cycle changes were determined using propidium iodide/flow cytometry based assay. KC cell lines with inducible *Klf5* knockdown were implanted as subcutaneous allografts in syngeneic immunocompetent hosts. *Klf5* knockdown was induced at Day 7 post-implantation. Tumors were monitored for 7 days and excised at Day 14 post-implantation. Immunohistochemistry (IHC) for KLF5, vimentin, and mouse CD107b (Mac-3) were performed. **Results:** Inhibitors targeting PI3K and MEK kinases were effective in reducing KLF5 protein levels in KC cell line. Knockdown and knockout of *Klf5* reduced cell proliferation and led to changes in cell cycle characterized by the reduction in the proportion of cells in G0/G1 phases and the corresponding increase in the proportion of cells in S phase. Subcutaneous allograft model showed significant decrease in tumor size after *Klf5* depletion. IHC staining of excised tumor show decreased KLF5 staining and loss of ductal architecture after *Klf5* depletion. Vimentin and Mac-3 staining showed increased fibrosis and macrophage infiltrate after *Klf5* depletion, respectively. **Conclusion:** KLF5 depletion reduces tumor growth in oncogenic KRAS-mediated mouse models of PDAC tumorigenesis. These results suggests that KLF5 may be an important mediator for KRAS-driven pancreatic carcinogenesis and a potential therapeutic target in pancreatic cancer.

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Spontaneous Skin Immune Dysregulation 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. Humans with WAS exhibit pathologies associated with heightened type 2 immune responses, including elevated serum IgE and treatment-refractory atopic dermatitis (AD). Though WAS patients develop severe AD, overt skin pathology has not previously been described in WASp-deficient mice. We found that mice lacking WASp have increased ear thickness, dermal and epidermal histopathological changes, and increased transepidermal water loss, indicating impaired physical skin barrier function. Consistent with this, we observed decreased epidermal expression of the tight junctional protein Claudin-1 in WASp-deficient mice. Impaired skin physical barrier function has been associated with changes in the skin microbiome and translocation of commensal microbes through the epithelium. Using 16S ribosomal sequencing, we found that the skin of WASp-deficient mice had significant enrichment of several bacterial species, including those from the Streptococcus genus, which may contribute to the observed skin pathology. Associated with the perturbations in skin physical barrier function and skin microbiome, we observed a significant increase in the number of CD45+ leukocytes in WASp-deficient mouse skin. Analysis of skin immune subsets revealed increases in multiple populations, including those associated with AD (basophils, eosinophils, group 2 innate lymphoid cells, and CD4+ T cells). However, we also observed homeostatic increases in the number of skin-dwelling γδ T cells, group 3 innate lymphoid cells, neutrophils, and CD11b+ monocytes/macrophages/DCs, suggesting that the inflammation is of a mixed phenotype. We next assessed the functional ramifications of the increase in skin immune populations using Luminex to measure protein expression of immune mediators. While many cytokines and chemokines were increased in WASp-deficient mouse skin, factors that showed the most upregulation were classical type 2- and 17-associated factors, including IL-4, IL-5, CCL-17, IL-17, and IL-23, while the type 1 signature cytokine IFN-γ was not detected. This perturbed cytokine milieu suggests coexisting type 2 and type 17 inflammation, which has been described in chronic cases of asthma and AD. We plan to use genetic and pharmacologic approaches to determine the relative contributions of type 2 and 17 cytokines to the development/maintenance of skin barrier dysregulation in WASp-deficient mice. We hypothesize that altered physical and immune barrier function may drive pathologies observed in WAS and may contribute to lack of patient response to standard AD therapy.

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Class I Histone Deacetylases Localize to Cardiac Myocyte Mitochondria and Contribute to Ischemia Reperfusion Injury

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Approximately half of the damage done to the heart by a myocardial infarction occurs during reperfusion of the ischemic region while the patient is in the care of the treatment team. While many different adjuvant treatments have been explored in an attempt to attenuate this ischemia-reperfusion (I/R) injury, little progress has been made in translating novel therapies to the clinic. Recently, it was discovered that epigenetic enzymes contribute to reperfusion-induced damage, but little is known about the exact mechanism by which they exacerbate I/R injury. Previously, we have shown that class I histone deacetylase (HDACs) activity acutely exacerbates I/R injury, and that inhibition of class I HDACs with MS-275 (entinostat) preserves left-ventricular (LV) function and substantially reduces the area of infarcted tissue in isolated rat hearts subjected to ischemia-reperfusion (IR) injury. Notably, this protective effect occurs whether MS-275 is given as a pretreatment or during the reperfusion phase alone. Given the acute nature of this protective effect, we hypothesized that class I HDACs mediate reperfusion injury by modulating the acetylation state of non-histone proteins in signaling cascades that are essential to cell survival. To examine this, hearts from male Sprague-Dawley rats were subjected to *ex vivo* I/R injury +/- class I HDAC inhibition during reperfusion. We then performed mass spectrometry to analyze the changes in the acetylome between sham and I/R groups with and without class I HDAC inhibition. Unexpectedly, mass spectrometry analysis revealed significant changes in the acetylation state of multiple mitochondrial enzymes. Further biochemical studies show that class I HDACs localize to cardiac mitochondria and may directly modulate mitochondrial acetylation and mitochondrial function during I/R injury. This study is the first to identify a class I HDAC that localizes to the mitochondria and emphasizes the importance of exploring class I HDAC inhibitors for protection against ischemia-reperfusion injury.

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Different Culture Conditions Alter Growth and Viability of Mesenchymal Stem Cells.

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Mesenchymal Stem Cells (MSCs) have been shown to exhibit a protective effect in many tissues. Specifically, their renoprotective effect in Acute Kidney Injury (AKI) shows promise in various treatment modalities. MSCs are difficult to study because they must be cultured from whole bone marrow for several passages to select for MSCs. Slow growth rates and high sensitivity to environmental stressors seen in MSCs, particularly those from aging individuals, necessitate careful consideration of culture conditions to make subsequent studies possible. **Objectives:** The purpose of this study was to evaluate a novel method for improving the survivability and viability of MSCs in cell culture using a partial exchange of media versus a complete change in media. **Methods:** Whole bone marrow was obtained from 5- or 12-month old male and female C57BL/6 mice and established in culture. At each culture change, partial exchange of media was done by removing two-thirds of the media and adding back the same volume of fresh media to the cells at each media change. Complete change of the media was done by removing the entire volume of media and replacing it fully with fresh media. Images were taken of the cell cultures at alternating three and four day intervals based on media changes and MSCs were counted using ImageJ. **Results:** Using partial change for MSC culture, we observed the growth and maintenance of MSC-like morphology over time was improved compared to MSC culture that was changed according to traditional full media change. MSC culture that underwent partial media change grew MSCs at a rate exceeding 18% faster than the culture that underwent whole media change. The culture undergoing partial media change was also able to sustain an average of 192% more MSCs than the culture undergoing whole media change. Lastly, cells undergoing partial media change were shown to resist senescence or failure to thrive for longer periods of time than cells undergoing whole media change. These differences were particularly striking for MSCs from aged individuals. **Conclusion:** This work contributes a methodology by which MSC cultures can be improved for the purpose of various analyses to better understand their protective effects. Future studies will look at the specific levels of various key growth factors to determine how the concentrations differ between these two media changing techniques and will determine the effect of these different regimen on maintenance of stem cell multipotentiality.

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Understanding How c-di-GMP Activates Transcription of Biofilm Genes in *Vibrio Cholerae*

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Biofilms pose a serious public health concern in both the medical and industrial setting. Their formation and persistence on catheters, pacemakers, sutures, and other indwelling medical devices account for over two million nosocomial infections and 100,000 deaths annually largely due to their significant decrease in susceptibility to antimicrobial agents. In the vast majority of bacterial species, the highly ubiquitous and important second messenger, cyclic dimeric guanosine monophosphate (c-di-GMP), is the central regulator of biofilm formation. More specifically, in *Vibrio cholerae (V. cholerae),* the causative agent of the life-threatening disease cholera and the recent devastating outbreak in Haiti, VpsR is the master Enhancer Binding Protein (EBP) that interacts with c-di-GMP to increase biofilm gene expression. Although EBPs typically activate RNA polymerase (RNAP) containing the alternate sigma factor, sigma54, substitutions at crucial residues in VpsR needed for EBP function suggest that the mechanism of VpsR activation is atypical and novel. Despite the abundance of data demonstrating c-di-GMP's positive regulatory role in gene expression, currently, the mechanism of c-di-GMP-dependent transcription activation is unknown. To address this knowledge gap and to determine the mechanism by which VpsR and c-di-GMP promote transcription, we first established an *in vitro* system. Using

RNAP containing the primary sigma factor, sigma70, we show for the very first time that c-di-GMP is sufficient to directly increase transcription of *V. cholerae* biofilms genes via VpsR by approximately 5-fold over basal level expression. Utilizing this system of RNAP, VpsR, and c-di-GMP, we have also determined protein-protein, protein-DNA interactions, and protein-second messenger interactions. While transcription activation requires c-di-GMP binding to a unique pocket in the AAA+-like domain of VpsR, VpsR functions as a dimer and binds the DNA with similar affinities with and without c-di-GMP. Using c-di-GMP and VpsR as a model for c-di-GMP-dependent gene expression, our findings present a new mechanistic paradigm in transcription activation. As c-di-GMP and EBPs are widely conserved in many bacterial pathogens, our studies with VpsR will not only lead to a general understanding of how this important second messenger regulates gene expression, but also provide us valuable insights in the development of new therapeutics targeting biofilm-based infections via c-di-GMP signaling.

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YTHDC2 Regulates Spermatogenesis through Promoting the Translation of N^6-methyladenosine-modified RNA

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Post-transcriptional modifications in RNA are present in all living organisms, and add an additional layer of control of gene expression and protein localization. Over 100 chemical modifications of RNA have been discovered; however, their functions are overwhelmingly undefined. Newly emerging as a critical post-transcriptional mRNA regulator is N^6-methyladenosine (m^{6}A), the most abundant internal modification in mammalian mRNA. m^{6}A is present on average ~3 times per mRNA and is necessary for mammalian life. It was recently characterized as a reversible, dynamic mark, which sparked extensive research on its function: m^{6}A has been shown to play critical roles in regulating stem cell pluripotency; controlling the circadian period; adipocyte differentiation; and fertility in mice. m^{6}A is recognized by selective binding proteins; YTHDF1 promotes the translation of m^{6}A-containing mRNAs, YTHDF2 expedites mRNA decay, and YTHDC1 affects the splicing of its targets. The biological function and RNA binding specificity of YTHDC2, another member of the YTH protein family, remains unknown. Using RNA affinity chromatography, gel shift assays, and UV Crosslinking Immunoprecipitation Sequencing (CLIP-seq), we report that YTHDC2 selectively binds m^{6}A along its consensus motif GGACU. Since YTHDC2 contains RNA helicase motifs, we hypothesized that it plays a role in translation. We fused N-YTHDC2 to a λ peptide (N-YTHDC2-λ), which binds with high affinity to Box B RNA, and used the construct in a tethering reporter assay. Tethering N-YTHDC2-λ to an mRNA reporter containing luciferase and five Box B domains (F-luc-5BoxB) increased translation efficiency 1.67-fold compared to tethering λ fused to an inactive linker sequence. However, N-YTHDC2-λ with an inactive helicase domain did not increase translation efficiency. Thus, YTHDC2 increases translation efficiency of its targets, likely through helicase activity. To determine how YTHDC2 may play a role in translation, we performed ribosome profiling of HeLa cells. We found that YTHDC2 associates with cellular fractions involved in translation initiation. Upon knocking down YTHDC2 using shRNA, we observed a robust decrease in the amount of mRNA localized to cellular fractions involved in translation initiation. Our data suggest that YTHDC2 plays a role in translation initiation. We created *Ythdc2* knockout mice, and found that they are infertile and have significantly smaller testes compared to those of littermates. In the testes, *Ythdc2* is temporally expressed as meiosis begins, and germ cells of Ythdc2 knockout mice do not develop past the spermatogonium stage. Altogether, our data suggest that YTHDC2 is an m^{6}A binding protein that plays essential roles in spermatogenesis by promoting translation initiation through helicase activity. YTHDC2 regulates spermatogenesis through promoting the translation of N^6-methyladenosine-modified RNA

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Iron-handling "MFe^{hi}" Macrophages In Adipose Tissue

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Macrophages are the predominant immune cell in adipose tissue (AT) and exhibit phenotypic variability depending on the obesity state of the tissue. Lean AT contains a population of anti-inflammatory "M2" AT macrophages (ATMs), whereas, obesity is associated with an influx of inflammatory "M1" ATMs. In contrast to M1 ATMs in obesity, the role of M2 ATMs in maintenance of a healthy lean state is relatively undefined. Our lab has previously published the discovery of the "MFe^{hi}" population of M2 ATMs, which has a two-fold increase in intracellular iron content and exhibits increased expression of genes involved in iron uptake (e.g. CD163), storage and release. This population composes 25% of M2 ATMs and exists in both lean and obese mouse and human AT. It remains unknown whether MFe^{hi} ATMs contribute to AT iron metabolism. To follow up on these studies, and to better understand how MFe^{hi} macrophages are regulating iron in AT, we provided variable iron through diets and by direct injection and predicted that they would compensate for excess iron to protect adipocytes from iron overload. We found that only MFe^{hi} macrophages took up the iron, and their gene expression corresponded with this uptake. Even with so much iron uptake by MFe^{hi} macrophages there was no change in iron content or gene expression in adipocytes, as hypothesized. Even though the MFe^{hi} ATMs change their gene expression in obese AT, we still observed that they are able to compensate for excess iron in the tissue. To determine the impact of MFe^{hi} ATMs on the systemic metabolic state, we aim to deplete MFe^{hi} ATMs *in vivo* in mice with cytotoxic CD163-targeted liposomes. Our preliminary studies with these liposomes have revealed CD163-targeted liposomes as a novel technique to specifically target CD163-expressing M2 ATMs. These studies will allow us to characterize the role of the MFe^{hi} population in maintaining healthy adipocyte iron concentrations and, thereby, insulin sensitivity. *Graduate Research supported by Vanderbilt MSTP NIH grant T32 GM007347-31 and F30 DK103438-02.*

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Unifying Pathway for Induction of Differentiation of Oligodendrocyte Precursor Cells

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Differentiation of Oligodendrocyte Precursor Cells (OPCs) to Oligodendrocytes via small molecules is a potential therapy for incurable diseases of demyelination such as multiple sclerosis. Oligodendrocytes are cells that make myelin which insulates neurons in the brain and spinal cord. In adults, OPCs can divide to maintain a stable pool of progenitor cells or differentiate into oligodendrocytes to allow for remyelination. However, in certain disease the OPCs do not differentiate, but continue to exist in their progenitor state. Therefore, in vitro drug screens have been performed to find small molecules which encourage OPCs to differentiate into oligodendrocytes. Surprisingly, the small molecules which perform the best in the screens for induction of differentiation have a wide variety assigned targets and don't seem to converge on any specific pathway. Similarly, research on differentiation induction by small molecules has mostly focused on each small molecule independently and its ability to induce differentiation attributed to its own previously ascribed mechanism. However, these fail to explain why some drugs with those targets induce OPC differentiation while different drugs with the same target do not. A unifying hypothesis to explain how widely different drugs with diverse targets can all lead to the same differentiation phenotype has not yet been proposed. Our work identifies a critical pathway for differentiation induction which is consistent across small molecule inducers of differentiation. Further, we have shown that this pathway is also critical for several small molecules previously ascribed different mechanisms of action in literature. Lastly, we have shown that this pathway is also engaged in vivo when mice are treated with small molecule inducers of differentiation. Our work describes a unifying hypothesis for differentiation induction via small molecules and illuminates a novel pathway for therapeutic targeting.

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Assessing MTH1 Inhibitors as a Novel Therapeutic Option for Ovarian Cancer Therapy

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Ovarian cancer is the most lethal gynecologic malignancy. While 60-80% of women respond to initial platinum based therapy, the vast majority of ovarian cancers relapse and become platinum resistant. Relapsed, platinum-resistant disease is considered incurable, with a median survival of one year. Although several second-line therapies are available, response rates are low (15-25%) and the identification of alternative treatment options is imperative. MTH1 inhibitors are a novel class of agents that target MutT Homolog 1 (MTH1), a non-essential protein in the Nudix hydrolase family. When MTH1 activity to remove oxidized nucleotides is inhibited, tumor cells incorporate oxidized nucleotides into DNA, resulting in activation of the DNA damage response and cell death. Importantly, cancer cells rely on the MTH1 enzyme for survival, whereas healthy cells do not. Accordingly, we investigated the role of MTH1 inhibitor in both platinum-sensitive and -resistant ovarian cancer. **Methods:** MTH1 inhibitor (TH588 and TH1579) activity was assessed in a panel of ovarian cancer cell lines and immortalized normal epithelium via clonogenic assays and immunofluorescence of 8-oxoguanine. TH1579 efficacy *in vivo* was assessed using intraperitoneal platinum-sensitive and -resistant ovarian cancer patient derived xenografts (PDXs). **Results**: MTH1 inhibitors TH588 and TH1579 demonstrated efficacy in ovarian cancer cell lines regardless of platinum sensitivity in colony forming assays, but did not impact the growth of immortalized ovarian surface epithelium or fallopian tube epithelium. Treatment of ovarian cancer cell lines A2780 and platinum-resistant A2780-CP200 with TH1579 resulted in increased 8-oxoguanine incorporation into DNA. Finally, TH1579 treatment resulted in significant reduction of tumor size in several ovarian cancer PDX models. **Conclusions**: MTH1 inhibitors TH588 and TH1579 demonstrate notable activity *in vitro* that is selective for ovarian cancer cell lines (relative to normal epithelium) and is observed regardless of platinum-sensitivity status. Additionally, TH1579 is a well-tolerated, orally available therapeutic agent with significant activity as a single agent in ovarian cancer PDX models. Collectively, these results warrant the further preclinical and clinical studies of MTH1 inhibitors in ovarian cancer.

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TLR4-mediated Epigenetic and Senescence Mechanisms in COPD

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Emphysema is a major component of COPD (chronic obstructive pulmonary disease), in which there is irreversible airspace enlargement and loss of vascular surface area. Previous work by our group has shown that TLR4 (Toll-like receptor 4) deficiency mice exhibit emphysema spontaneously as they aged, which was due to an oxidant/antioxidant imbalance. Reduced expression of TLR4 has been found to associates with more severe emphysema and airflow obstruction in human emphysema. However, the mechanisms of these effects remain poorly defined. Here we demonstrate that TLR4 is highly expressed in mouse lung endothelium as well as in human lung, and endothelial TLR4 transgenic mice, crossed with TLR4 -/- mice (Tie2-TLR4 tg X TLR4 -/-) resulted in complete prevention of emphysema. Interestingly, epithelial TLR4 transgenic mice, crossed with TLR4 -/- mice (CC10-TLR4 tg X TLR4 -/-) demonstrated partial prevention of emphysema but the mechanisms appear to be distinct from that of endothelial-targeted TLR4 transgenic mice. Moreover, conditional, endothelial-specific silencing of TLR4 in wild type mice led to emphysema, suggesting that the endothelium was a key compartment. We found that the senescence-associated gene, $p16^{INK4A}$, is highly expressed in TLR4 -/- mice lungs as well as in human COPD lungs. Endothelial cells from TLR4-KO mice lungs (MLEC) or TLR4 silencing in hLMVEC (human lung microvascular endothelial cells) also resulted in increased $p16^{INK4A}$ transcriptional activity and $p16^{INK4A}$-dependent changes in cell proliferation. These effects involved TLR4-dependent changes in HDAC2 expression, resulting in increased $p16^{INK4A}$ via histone acetylation of the $p16^{INK4A}$ promoter region. These finding provide critical insights into previously unrecognized links between innate immune signaling and HDAC2-$p16^{INK4A}$ – mediated cellular senescence in emphysema.

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LIN52 Stability and Formation of Cell Cycle Gene Regulators, the DREAM and MMB Complexes

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Cell cycle events are normally precisely timed and tightly coordinated. Cell cycle deregulation is, in turn, a hallmark of cancer. The DREAM (DP, RB-like, E2F, and, MuvB) and MMB (Myb-MuvB) complexes are important for maintaining appropriate cell cycle gene expression. DREAM plays a role in repressing 800 cell cycle genes during G1 or G0 (quiescence), and the MMB complex contributes to increased G2/M gene expression, required for cell division. LIN52 is part of the MuvB core of proteins, shared in both of these complexes, and is necessary for their assembly and function. Data from The Cancer Genome Atlas reveal that deletions in the *LIN52* gene are associated with decreased survival in some cancers. The mechanism and cellular consequences of LIN52 alterations, however, are unknown. Here, we investigated the mechanisms controlling the degradation of LIN52 and how it may be influence the formation of DREAM and MMB. DYRK1A (dual-specificity tyrosine-regulated kinase)-mediated phosphorylation of LIN52 at the serine-28 residue (S28) is critical for LIN52 binding to p130 and, in turn, DREAM formation. We noted that LIN52 protein levels are increased when it cannot be phosphorylated due to a loss or inhibition of DYRK1A, or when S28 was replaced with alanine (S28A). Ectopic expression of either LIN52-V5 or LIN52-S28A proteins resulted in a dramatic downregulation of native LIN52, suggesting that LIN52 levels are tightly regulated in the cell. Our results support proteasome-mediated degradation of LIN52 following its phosphorylation by DYRK1A. Specifically, cells treated with cycloheximide showed a progressive decrease in LIN52 levels that was reversed by co-treating those cells with the proteasomal inhibitor, MG-132. We also found evidence of LIN52 ubiquitination using mass-spectrometry and Western blotting. Furthermore, harmine inhibition of DYRK1A, loss of DYRK1A expression, or S28A mutation all resulted in increased stability of LIN52. Using RT-qPCR, we observed that LIN52 mRNA levels slightly increased upon loss of DYRK1A activity and decreased when recombinant LIN52 was overexpressed, suggesting that transcriptional regulation could play a role in control of LIN52 levels. However, the differences in protein levels between LIN52-V5 and LIN52-S28A mutant, despite equivalent mRNA expression, could be only explained by protein-level regulation. Altogether, these results suggest DYRK1A-mediated phosphorylation of LIN52 may lead to LIN52 degradation by the proteasome. These observations place LIN52 in a position to influence the formation of the DREAM and MMB complexes and, in turn, impact cell cycle regulation. The potential role of LIN52 stability in influencing transitions between DREAM and MMB will be discussed.

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The Role of CD70 in Vascular Function

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We have recently shown that CD70 is important in formation of effector memory T (T_{EM}) cells in hypertension and T_{EM} cells enhance the hypertensive response and end organ damage caused by repeated hypertensive stimuli. CD70 has traditionally been identified on antigen presenting cells and interacts with CD27 on T cells, promoting proliferation and memory cell formation. The precise cell types that express CD70 and the role of CD70 on non-immune cells has not previously been investigated. Here we discovered a new role of CD70 in vascular function. Studies of mesenteric vascular reactivity showed that $CD70^{-/-}$ mice have markedly impaired endothelium-dependent vasodilatation to acetylcholine compared to WT mice (44± 3 vs 25 ± 4 %) at baseline. In contrast, there were no differences in relaxation responses to sodium nitroprusside. Pre-incubating vessels with the endothelial nitric oxide synthase (eNOS) inhibitor L-NAME blocked endothelium-dependent vasodilatation to acetylcholine in WT mice by 50%, in contrast, L-NAME had no effect on endothelial vessel relaxation in $CD70^{-/-}$ mice. This suggests that vessels of $CD70^{-/-}$ mice lack the capacity to produce nitric oxide. Western blots for the endothelial nitric oxide synthase (eNOS) showed a marked reduction of this enzyme in the aortas of $CD70^{-/-}$ as compared to wild type mice. In additional experiments, we showed that human umbilical vein endothelial cells express CD70 mRNA and that this is increased by > 30 fold by laminar (15 dynes/cm^2) compared to oscillatory shear. Finally, using immunohistochemistry, we identified CD70 protein localized to resistance vessels of the kidney of ang II-treated mice. These data define a new role of CD70 in modulating vascular function. Expression of CD70 might contribute to the previously identified ability of endothelial cells to present antigen to T cells and promote T cell activation and T_{EM} cell formation. Moreover, CD70 seems to be linked to eNOS expression by mechanisms that remain to be defined.

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Epigenetic Regulation of Imprinted Gene *Grb10* during Neuronal Differentiation

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The brain has emerged as a main target of genomic imprinting - an epigenetic phenomenon in which genes are expressed from a single parental allele and controlled by differentially methylated *cis*-acting regulatory elements, termed imprinting control regions (**ICRs**). Imprinted genes play central roles in neurodevelopmental processes, including neurogenesis, neural migration, proliferation and differentiation. Indeed, dysregulation of imprinted gene expression in the human brain is strongly associated with neurological or behavioral disorders. The imprinted gene, *Grb10,* encodes a critical signal adaptor protein and is a novel model for tissue-specific gene regulation during neurodevelopment. In embryonic stem cells and non-neuronal somatic tissues, *Grb10* is expressed from the major promoter on the maternal allele. Intriguingly, in neurons, *Grb10* is expressed exclusively from a minor promoter on the paternal allele. The methylation status of the ICR of both alleles remains

static and the epigenetic mechanism controlling this allelic and promoter switch in *Grb10* expression during neurodevelopment is unknown. I hypothesize that regardless of methylation status at the ICR, architectural chromatin binding protein, **CTCF**, binding at the ICR plays a role in controlling neuronal *Grb10* expression. To test my hypothesis, I will generate CTCF binding site mutations at the *Grb10* ICR using CRISPR in mouse embryonic stem cells (**mESCs**) that will then be differentiated into motor neurons. Cultured motor neurons express significant levels of paternal neuronal *Grb10* and are a readily available system to study epigenetic changes throughout neuron differentiation. I will also analyze the functional effects of putative downstream enhancers (including novel CTCF binding regions, **NCBR**) on the promoter switching at the *Grb10* locus using CRISPR in the mESC-motor neuron system. Together, these experiments will identify interactions between CTCF, DNA methylation and distal enhancers during neuronal commitment, and possibly uncover a novel mechanism governing the regulation of imprinted genes. Importantly, the information gained from these experiments may inform the epigenetic regulation of non-imprinted genes in the mammalian genome during cell differentiation. By understanding the neuroepigenetic mechanisms required to coordinate the complex changes in gene expression during cell differentiation, we can begin to address disease states and possible therapies in the brain.

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Identification of Bacterial Genes Required for *Rickettsia parkeri* Infection

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Rickettsia parkeri is an obligate intracellular bacterial pathogen that hijacks the host cell actin cytoskeleton to facilitate infection. *R. parkeri* infection involves three major steps: host cell invasion, intracellular growth, and formation of actin tails to promote cytosolic motility and cell-to-cell spread. To better understand the molecular mechanisms that underlies these processes, this study aimed to determine the bacterial genes required for *R. parkeri* infection and provide a better understanding of the life cycle of this poorly characterized human pathogen. To identify genes important for *R. parkeri* infection, we conducted a transposon mutagenesis screen and generated a library of 108 transposon mutants that caused a small plaque phenotype. Transposon insertion sites for each small plaque mutant were then mapped using polymerase chain reaction (PCR) amplification and DNA sequence analysis. We have thus far identified genes involved in invasion, intracellular replication, actin tail formation and metabolism. Secondary immunofluorescence-based screening was done to determine if specific steps of the infectious life cycle were targeted, including invasion, actin-based motility and spread. Based on these analyses, we found mutant Sp116 to be defective only in cell-to-cell spread and chose to further explore how it regulates infection. The Sp116 mutation lies within a gene coding for the *Rickettsia* ankyrin repeat protein (RARP), an effector similar to one that was previously shown to be secreted by other *Rickettsia* species. Thus, we hypothesize that RARP interacts with host factors to specifically facilitate spread. Importantly, we have complemented the Sp116 spread defect by exogenously expressing wild-type RARP, confirming that the spread defect is caused by a mutation in the *rarp* gene. Future experiments will focus on exploring the molecular mechanism of RARP by determining its subcellular localization and characterizing its potential host protein partners. Most mutants from this screen remain unexamined and additional efforts will continue to expand upon and address their specific roles in host cell infection and manipulation. The results of this ongoing study will help facilitate research into novel regulatory mechanisms utilized by this pathogen and develop therapeutic interventions to control bacterial infection.

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Differential Biomechanical Regulation of Endothelial CD39 from Vein Grafts by Pulsatile Radial Forces

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Human saphenous veins are the most frequently used bypass grafts in coronary and peripheral artery bypass surgery, but are disproportionately prone to failure when compared to arterial grafts due to adverse remodeling. The endothelium senses radial and linear biomechanical forces exerted by blood flow. While thick-walled arteries are subjected to pulsatile strain, thinner-walled venous blood flow *in situ* is non-pulsatile until transposed as an arterial graft. The impact of cyclic stretching from pulsatile distention on the vein wall is not well elucidated. CD39 is a potent ecto-enzyme at the endothelium:blood interface regulating blood fluidity and suppressing vascular thrombo-inflammation by enzymatically dissipating extracellular ATP and ADP, and facilitating adenosine generation. We recently identified that endothelial CD39 expression can be induced by linear biomechanical forces (laminar shear stress). However, the response of CD39 to cyclic stretch during vein graft arterialization remains unknown. We *hypothesized that the vasculo-protective enzyme CD39 is induced by the venous endothelium in response to radial forces associated with pulsatile stretch.* **Methods:** Primary human saphenous vein endothelial cells (HSVEC) were isolated from vein remnants from patients undergoing coronary bypass surgery, and exposed to low and high levels of cyclic stretch *in vitro*, mimicking arterial and vein graft patterns using flexible membrane-bottomed plates. *Cd39* gene and protein expression were quantified by qRT-PCR and immunoblot. Ecto-ATPase and -ADPase activity of CD39 on cultured cells following cyclic stretch was quantified with a malachite green assay. Mice with a haploinsufficiency of *Cd39* (*Cd39*$^{+/-}$) underwent carotid bypass surgery with a vein segment as an interposition graft. Subsequent neointimal hyperplasia was compared with similar grafts in wild-type controls. **Results**: High (16%, 1 Hz) and low (5%, 1 Hz) levels of cyclic stretch of HSVEC elicited similar morphologic realignment of cells perpendicular to the direction of stretch, but distinct patterns of CD39 expression. *Cd39* transcript remained unchanged following 24 or 48 hours of low or high stretch (n=6, each). Low-level cyclic stretch did not alter CD39 protein expression after 24 or 48 hours compared with static controls (n=6, each). In stark contrast, cyclic stretch at 16% induced a 130% increase in CD39 protein expression after 48h, and 60% increase after 24h compared with static controls (n=6 each, $p < 0.05$). We observed corresponding increases of 60% and 100% in CD39-dependent enzymatic hydrolysis of ATP and ADP, respectively by HSVEC following high levels of stretch (n=4, $p < 0.005$). *In vitro*, cyclic stretch did not induce transcripts of other ectonucleotide-degrading enzymes. Preliminary studies in a murine model of vein graft disease suggest that CD39-haploinsufficiency exacerbates neointimal hyperplasia

in isogenic vein-to-artery grafts compared to wild-type controls. **Conclusions**: We have identified CD39 as a novel, stretch-responsive endothelial ectoenzyme in human saphenous veins, which may represent a protective response to venous distention from arterial stretch patterns.

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Microvascularization of Grade I Meningiomas: Effect on Tumor Volume, Blood Loss, and Patient Outcome

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Quantitative assessment of tumor microvascularity has the potential to improve prognostication, advance understanding of tumor biology and help narrow potential molecular therapies. While the role of tumor microvascularity has been widely studied in meningiomas, we sought to examine both the role of automated measurements and the impact on surgical outcome. **Methods**: Patients Grade I meningiomas (n=207) were operated on between 1996 and 2011 were analyzed for measures of microvascularity. Manual methods and computational analysis of the microvascular patterns by means of fractal analysis was performed. **Results:** The mean age of the patients was 55.4±14.8 years, and 63 (30.4%) were male. For tumor sizes ≥3 cm, patients were significantly older, more frequently male, and had greater EBL, greater tumor volume, higher MIB index, higher vWF, lower HIF-1 expression, and worse OS. In a multivariate logistic regression, MIB (OR=1.14, p=0.05), vWF (OR=1.01, p=0.01), and HIF-1 (OR=1.54, p=0.0001) significantly predicted tumor size. In a univariate linear regression, although multiple factors were predictive of EBL, only vWF remained significant in a multivariate analysis (β=0.39, p=0.004). Lastly, MIB was useful via Kaplan-Meier survival analysis in predicting patients with disease progression where an MIB cutoff of ≥3 (sens 36%, spec 82.5%) predicted disease progression; a MIB ≥3 showed significantly shorter mean PFS (140.1±11.7 vs. 179.5±7.0 months, log rank test, p=0.05). A Cox proportional hazards model showed a trend for MIB in predicting disease progression in a hazards model (OR: 1.08; 95% CI: 0.99, 1.19; p=0.08). **Conclusions**: Our results support the importance of various microvascularity measures in predicting preoperative (e.g., tumor size), intraoperative (e.g., EBL), and postoperative (e.g., PFS, OS) outcomes in patients with grade I meningiomas. A MIB cutoff of 3 showed good specificity for predicting tumor progression. Various measures to detect aberrant tumor microvasculature differed, possibly reflecting the heterogeneous underlying biology of meningiomas.

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Toll Like Receptor 4 Contributes to Drug Reward Behavior and Synaptic Physiology

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Substance use disorders remain significant problems in society. Drugs of abuse alter excitatory synapses within the nucleus accumbens (NAc) suggesting a role in dependence/addiction. The NAc is primarily made up of medium spiny neurons (MSN) that express either D1 or D2 dopamine receptors. These cells belong to distinct circuits that differentially modulate reward behavior. Neuron-mediated regulations of excitatory synapses within the NAc in response to drug experience are well-studied. Evidence also suggests the involvement of the brain's innate immune system in modulating motivated behavior and synaptic physiology. More specifically, pharmacologic manipulation of toll-like receptor 4 (TLR4), a pattern-recognition molecule of the innate immune system, attenuates cocaine-reward learning. In the brain, TLR4 is primarily associated with phagocytes with the ability to regulate synaptic physiology. Despite these observations, mechanistic examination of how the innate immune system contributes to NAc synaptic physiology in relation to drug-reward behavior has not been pursued. We hypothesize that cocaine experience remodels excitatory synapses within the NAc in a TLR4-dependent manner. **Methods:** We used a combination of behavioral and *ex vivo* slice electrophysiology assays on wild type (WT), TLR4 knockout (TLR4.KO), and microglial TLR4.KO (mTLR4.KO) mice. We performed cocaine-induced locomotor sensitization and conditioned place preference (CPP) assays to test drug reward learning. Open field and novel object recognition tests were used to control for basal locomotor activity, anxiety, and working memory. To examine synaptic function, we performed cell-type specific voltage-clamp recordings from NAc MSNs in WT, TLR4.KO, and mTLR4.KO mice under naïve and cocaine-treated conditions. **Results:** TLR4.KO animals exhibit significant attenuations in cocaine locomotor sensitization and cocaine CPP. Importantly, there were no differences in basal locomotor activity, anxiety, or working memory. mTLR4.KO animals exhibit a deficit in acquisition of cocaine locomotor sensitization. *Ex vivo* slice electrophysiology revealed weaker synaptic strength in TLR4.KO animals in both D1 and D2 MSNs. Additionally, we found altered synaptic strength on D2 MSNs in TLR4.KO mice following drug experience. **Discussion:** Alterations of the reward system are a characteristic of many neuropsychiatric conditions including substance use disorders. Despite the high prevalence and cost of such disorders, effective treatments are lacking in part due to an incomplete understanding of the underlying pathology. We used drug reward assays and transgenic animal models to study NAc synaptic physiology and related behaviors in a TLR4 dependent manner. We found that TLR4 participates in reward behavior and shaping of NAc physiology. Future experiments will determine signaling mechanisms which may uncover novel therapeutic targets to mitigate neuropsychiatric conditions.

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Mediawiki as a Platform to Provide Readily Available Information on Research Residency Programs

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While many residencies use the internet platform to present program information to potential applicants, the content displayed still leaves many applicant questions unanswered. A cursory literature review reveals that the accessibility of residency program information, despite the use of web technologies, is limited, and many websites of certain specialties, including pathology, general surgery, and otolaryngology, lack enough information for applicants to make effective decisions when applying. Although the American Medical Association has constructed a database of residency programs to help fill this gap, a similar resource for research-focused residencies for those pursuing a career as a physician scientist does not exist. Because the most accurate information can be provided by program officials and residents, we utilized the MediaWiki platform. This allows programs to directly submit pertinent information into a central repository that becomes navigable by future residents and fellows. Here we present the feasibility of using the MediaWiki platform for creating such a resource and our experience in having programs submit their own information. **Methods**: The research residency wiki was established in 2015 by installing MediaWiki software on a LAMP (Linux, Apache, MySQL, and PHP) server stack through Linode, a virtual private server (VPS) provider. Following installation two plugins were added: (1) VisualEditor, which allows editing wiki pages with a graphical user interface, and (2) ConfirmEdit, which prevents spam bot activity. The platform is open in regards to account creation. Templates were provided for residency programs to use when providing information and contact information to allow us to assist. **Results**: At this point, the residency wiki has been running for approximately 1.5 years; and, to date, we have had 52 programs submit their information into the wiki. Additionally, we have had several programs state that their trainees utilized the database when applying for residencies. **Discussion**: Our attempt to establish a central location for applicants to find research residency program information has been met with relative success. Although additions to the wiki are still ongoing, several programs have already contributed their information to the system. The MediaWiki platform has provided several advantages during construction, including ease of use, open access to both interested programs and students, and powerful internal search capabilities allowing visitors to easily find items of interest. Also, while successful addition of programs to the residency wiki has been steady, the slowest steps in program addition is the contribution of information by residency programs. Increasing use and publicity of the database has helped increase the rate at which programs are added, however, improvements are still needed to fill the wiki to a robust capacity.

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Ferroptosis Activation Contributes to Traumatic Brain Injury Pathophysiology

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Traumatic brain injury (TBI) is a common health problem associated with significant financial cost and long-term disability. Despite the burden of TBI, mechanisms of neuronal death remain unclear. TBI triggers multiple pathways of neuronal death, possibly including ferroptosis – a newly discovered cell death program that is switched-on by the discoordination of two major redox systems: thiols (glutathione peroxidase 4 (Gpx4)/glutathione (GSH)) and lipid peroxidation. Gpx4 is essential for life due to its unique ability to reduce phospholipid hydroperoxides (PL-OOH) to phospholipid hydroxides (PL-OH) utilizing GSH as a reducing substrate. Insufficiency of this system through GSH depletion or Gpx4 inactivation results in cell death due to the accumulation of PL-OOH and diversified cleavage products. More specifically, we have shown that 12/15-lipoxygenase (LOX) mediated oxidation of arachidonic (AA) and adrenic (AdA) acid-containing phosphatidylethanolamines (PE) is a key regulatory step in ferroptosis execution. We have shown that TBI leads to loss of GSH and accumulation of lipid oxidation products in cerebrospinal fluid (CSF) indicating clinical relevance of this pathway; however the role of ferroptosis in acute brain injury has not been evaluated. We hypothesized that ferroptosis is activated after TBI, and suppression of PE oxidation via 12/15-LOX inhibition confers neuroprotection. **Methods**: Mouse hippocampal neurons were exposed to pro-ferroptotic insults (RSL3, BSO, erastin, glutamate) or *in vitro* TBI (mechanical stretch). Adult C57/B6 mice underwent controlled cortical impact (CCI). GSH levels (ThioGlo assay) and expression of Gpx4 and 12/15-LOX (Western Blot) were measured in CCI vs naïve (n=4/group). Changes in PE oxidation were measured in cortex, hippocampus, and plasma from naïve and injured animals (liquid chromatography tandem-mass spectrometry (LC-MS/MS)). Effects of early (10 min) and late (24 hr) baicalein (12/15-LOX inhibitor) administration post-CCI on cognitive (Morris water maze (MWM)) outcomes were evaluated in CCI+inhibitor, CCI+vehicle, and sham groups (n=9-10/group). **Results:** Pro-ferroptotic insults and *in vitro* TBI caused neuronal death (LDH release) rescued by baicalein, ferrostatin-1, and liproxstatin-1 (lipid radical-trapping antioxidants) administration. CCI resulted in significant PE peroxidation, with peak oxidation at 1 hr post-injury and subsequent attenuation at 4 and 24 hr. Major products included 15-hydroperoxy eicosatetraenoic acid (HpETE) and 17-hydroperoxy docosahexaenoic acid (HpDHA), indicating an increase in 15-LOX activity. Expression of 15-LOX2 significantly increased at 1 hr post-injury and remained elevated at 4 and 24 hr in ipsilateral cortex and hippocampus. GSH levels were significantly depleted and Gpx4 expression unchanged at 4 and 24 hr post-CCI. Administration of baicalein both early (10 min) and delayed (24 hr) post-injury improved MWM performance, as measured by acquisition of spatial learning on days 10-15 vs vehicle. **Conclusions**: TBI results in thiol and lipid disruptions consistent with ferroptosis activation. Inhibition of 12/15-LOX and other major regulatory pathways of ferroptosis confers neuroprotection in our CCI model. *Support: NS061817;NS076511*

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Krüppel-like Factor 5 Regulates Regeneration of Intestinal Stem Cells Following Gamma-Irradiation Injury

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Ionizing radiation (IR) is a commonly used cancer therapy that targets actively proliferating cells. The intestine is particularly vulnerable to IR damage due to its proliferative nature, making gastrointestinal side effects inevitable. The intestinal epithelium has an enormous self-renewal capacity maintained by "active" intestinal stem cells (ISCs) that express LGR5 (Leucine-rich repeat containing G protein-coupled receptor) and reside at the base of the crypts. $LGR5^+$ ISCs are sensitive to IR damage, yet diphtheria toxin receptor-mediated targeted ablation of these cells result in failure of regeneration. Krüppel-like factor 5 (KLF5) is a zinc-finger transcription factor expressed in proliferating cells, including $LGR5^+$ ISCs, of the intestinal epithelium and is involved in regulation of cell proliferation and differentiation. We observed that KLF5 is expressed in the majority of the $MKI67^+$ proliferating cells in mice undergoing intestinal regeneration following total-body g-irradiation (TBI) injury. However, the role of KLF5 in post-IR regeneration has not been elucidated. **Methods and Results**: *Lgr5*-EGFP-IRES-Cre^{ERT2} mice, $Rosa^{tdTomato}$ mice, and $Klf5^{fl/fl}$ mice were crossed to generate $LGR5^+$ cell lineage-traceable transgenic mice with or without inducible-conditional knockout of *Klf5* ($Lgr5^{Ctrl}$ and $Lgr5^{\Delta Klf5}$). Tamoxifen was administered for 5 consecutive days to induce recombination in $LGR5^+$ cells in $Lgr5^{Ctrl}$ and $Lgr5^{\Delta Klf5}$ mice. Mice were then exposed to 12 Gy TBI and injected with EdU 3 h prior to euthanasia. Immunofluorescent staining was used to examine the lineage tracing, proliferative, and apoptotic status of the $LGR5^+$ ISCs and lineages. Generally, post-irradiation intestinal regeneration is divided into two phases: the apoptotic phase during 0 to 48 h, followed by the regenerative phase during 48 to 96 h post-TBI. During homeostasis in the absence of TBI, *Klf5* deletion resulted in a slowing of proliferation and lineage tracing from $LGR5^+$ cells from 0 to 96 hours following tamoxifen treatment, as indicated by the decreasing number of EdU-incorporated cells. This suggests that *Klf5* deletion does not completely inhibit proliferation immediately after its deletion. In TBI-treated $Lgr5^{Ctrl}$ mice during the apoptotic phase, $Lgr5^{\Delta Klf5}$ mice showed increased number of apoptotic LGR5 lineages at an early time point and throughout the phase compared to $Lgr5^{Ctrl}$ mice. This indicates that KLF5 regulates DNA damage-induced cell death by regulating the early DNA damage response. Furthermore, LGR5 lineages repopulated intestinal crypts during the regenerative phase, as indicating by enlarged crypts that are entirely comprised of $tdTomato^+$ cells. However, deletion of *Klf5* abrogated the regenerative capacity, as shown by the significantly diminished number of $tdTomato^+$ cells in $Lgr5^{\Delta Klf5}$ mice. **Conclusion**: Taken together, these data indicate that KLF5 modulates the regenerative response of the intestinal epithelium through functions beyond the role of a proliferation regulator in $LGR5^+$ ISCs and lineages.

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Using Metagenomics to Monitor the Lung and Gut Microbiome in Critically Ill Children

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Interactions between the host's immune system and the microorganisms that make up the microbiome are important to a patient's health and disease. While alterations of the bacterial microbiomes of the respiratory and intestinal tracts have been implicated in a wide range of diseases, and are associated with the progression of critical illness, there is little literature about the microbiome in critically ill children, and even less is known about the viral and fungi microbiomes. Fortunately, continued advances in metagenomics have enabled the reliable determination of the bacterial, fungal, and viral constituents of a patient's microbiome in a single sequencing experiment. **Aim:** To determine the complete bacterial, viral, and fungal microbiome in a population of critically ill children. Secondary aims are to assess its stability and resilience in a broad clinical context, to determine the importance of microbiome changes that occur early in a patient's life, and to detail the rate of microbiome recovery following acute disease. **Hypothesis:** Our hypothesis is that there are differences in the lung and intestinal microbiomes of children admitted with acute infections when compared to children that are admitted to the ICU following acute trauma or elective surgery. We hypothesize that the microbiome will be notably affected by antibiotic administration, mechanical ventilation, and disease progression. Furthermore, while some of these forces may drive changes in both the intestinal and lung microbiota, we expect to witness situations in which only one or the other are affected. Finally, we hypothesize that a patient's microbiome may not fully return to baseline following these perturbations. **Methods:** Children admitted to the Connecticut Children's Medical Center pediatric ICU will be approached for enrollment in this study. Those enrolled will have stool samples collected while an inpatient and following discharge, and sputum will be collected at the time of intubation or admission and then twice weekly while the child is mechanically ventilated. We, in collaboration with Jackson Laboratories, will use metagenomic sequencing to detail both the lung and intestinal microbiomes' taxonomic diversity, the abundance of well-known pathogenic organisms, and the proportion of sequencing reads aligning to bacterial, viral, and fungal genomes. We will then (1) assess the changes that occur in a patient's microbiome after admission to the pediatric ICU, (2) detail the specific antibiotics and therapeutic interventions, on a per-patient basis, that correlate with these changes, (3) compare the composition of the organisms within the lung to those of the gut, and (4) determine the rate of microbiome community recovery following critical illness.

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ETV6 Represses *Pax5* in Early B Cell Development

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Recent studies from our group and others have revealed a role for *ETV6* germline mutations in predisposition to ALL. Although ETV6 is among the most commonly mutated genes in ALL, its mechanistic role in leukemogenesis remains unclear. ETV6 is an ETS family transcription factor, and the germline mutation (P214L amino acid change) identified by our group and others impairs the transcriptional activity and nuclear localization of ETV6 in a dominant negative fashion. The goal of this project is to determine the role of ETV6 in early B cell development and define how germline *ETV6* mutations result in predisposition to leukemia. To identify functions of ETV6 in B cell development, we queried the gene expression commons database for evidence of Etv6 expression during B cell development, and found that *Etv6* is highly expressed in hematopoietic stem and lymphoid progenitor cells through the pre-pro-B stage (FrA), but its expression is significantly reduced in fraction B and thereafter. We confirmed Etv6 expression decreases as B cells develop and is negatively correlated with *Pax5* expression in mouse cells (r^2=.9993; P= 0.0167). We then found that *ETV6* expression was higher in the early B cell fractions compared to the preB cell fraction in patient samples, while *PAX5* expression was higher in the preB cell fraction compared to the early B cell fraction. Next, we overexpressed an empty vector (MiG), wild type (WT) ETV6 and ETV6 P214L in a murine lymphoid progenitor line (Ba/F3). ETV6, but not ETV6 P214L overexpression significantly decreased *Pax5* expression (P≤0.05). We then measured the association of ETV6 with putative ETS factor binding sites within the *Pax5* transcription start site (TSS) using ChIP-PCR. ETV6 is associated with the proximal GGAA site 72 base pairs upstream of the *Pax5* TSS, but not GGAA sites further from the TSS. In addition, the transcriptional repressors SIN3A and HDAC3 were detected on the same regions of the *Pax5* locus. We next determined the consequences of ETV6 mutation on the recruitment of ETV6, SIN3A, and HDAC3 to the *Pax5* locus by performing ChIP-PCR in Ba/F3 cells. We detected association of ETV6, SIN3A and HDAC3 with the proximal GGAA site upon expression of WT ETV6, but not ETV6 P214L. We conclude that ETV6, SIN3A and HDAC3 are responsible for the repression of *Pax5* transcription. To determine the consequences of ETV6 P214L expression on B cell development, we generated a transgenic mouse expressing the P214L mutation in the endogenous ETV6 gene. Preliminary data suggests that these mice are thrombocytopenic, similar to patients with germline ETV6 mutation. In conclusion, ETV6 regulates *Pax5* expression through the recruitment of SIN3A and HDAC3 to the *Pax5* locus. These findings are significant because *Pax5* misregulation results in a B cell development halt, lineage infidelity and leukemogenesis.

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Cytokine Levels in Bronchoalveolar Lavage Specimens From E-cigarette Users, Smokers, and Nonsmokers

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Traditional cigarette smoke increases lung inflammation and the expression of certain proinflammatory cytokines, but the effect of e-cigarettes is unknown. This pilot study investigated the levels of pro-inflammatory cytokines IL-6, -8, and TNF-alpha, and the anti-inflammatory cytokine IL-10 from bronchoalveolar lavage (BAL) specimens from cigarette users, nonsmokers, and e-cigarette users. **Methods:** Five e-cigarette users, 10 non-smokers, and 8 cigarette smokers underwent BAL. Tobacco-user status was verified using a NicAlert test and the Fagerstrom Test for Nicotine Dependence. Smokers used at least 10 cigarettes per day for at least six months. E-cigarette users did not smoke a traditional cigarette for the past year and used e-cig daily for at least three months. Non-smokers used fewer than 100 cigarettes in their lifetime and neither cigarettes nor e-cigarettes in the past year. Cytokines were measured using the MSD Multi-Spot Assay System. **Results:** The trend of cytokines IL-10, IL-8, and IL-6 levels were lower in e-cig users than smokers by 33%, 10%, and 48%, respectively. These levels were higher in e-cig users than nonsmokers by 5%, 18%, and 71%, respectively. TNF-alpha levels were comparable between e-cig users and smokers, and 16% higher in e-cig users than nonsmokers. None of these differences were statistically significant. **Conclusion:** While no statistically significant differences were seen in this small pilot study, 2 of 3 inflammatory cytokine levels from BAL specimens of e-cig users were lower compared to smokers, and all three were higher compared to nonsmokers. The anti-inflammatory cytokine IL-10 in e-cig users was lower than in smokers, and similar to nonsmokers. We have demonstrated that these select cytokines in smokers and e-cig users are both elevated compared to nonsmokers, though an adequately powered study is needed to determine if actual differences are present.

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Gene Fusion Analysis of Pdr1-Regulated Gene Expression in Anti-Fungal Drug Resistance

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Candidemia represents the third most common bloodstream infection in humans. *Candida glabrata* is the second leading fungal pathogen associated with this infection and exhibits high frequency resistance to azole drugs that constitute the major clinical antifungal agent. The number of clinically effective anti-fungal drugs is small; therefore our lab aims to understand the mechanisms that lead to the resistance in order to better treat patients. In *C. glabrata*, the primary cause of azole resistance is mutations in a transcription factor-encoding gene called *PDR1*. These mutant forms of Pdr1 behave as hyperactive positive transcriptional regulators and overexpress genes under Pdr1 control. Our lab has identified 25 different genes that contain Pdr1 binding sites called Pdr1 Response Elements (PDREs). I have constructed gene fusions between these PDRE-containing genes and a bacterial gene encoding an easily assayable protein called beta-galactosidase as a means of measuring the extent of Pdr1 control of many of these loci. I have initially tested 14 different candidate genes and demonstrated that 7 of these are clearly responsive to genetic changes that alter Pdr1 activity levels. My experiments will provide new tools and insight into Pdr1-regulated gene expression in *C. glabrata.*

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Characterizing Immune Responses in Nevirapine-induced Severe T-cell Mediated Adverse Drug Reactions

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Nevirapine is used globally and is effective in the combination treatment of human immunodeficiency virus (HIV). Its current use in first-line therapy is limited by severe immune-mediated adverse drug reactions (IM-ADRs) that significantly impact patient outcome and inflict substantial cost on healthcare. Stevens-Johnson Syndrome/ Toxic epidermal necrolysis (SJS/TEN) is the severest IM-ADR caused by nevirapine. SJS/TEN is a class I restricted, CD8+ T-cell dependent hypersensitivity syndrome, which presents as a severe blistering skin rash that can result in extensive epidermal necrosis. SJS/TEN is often complicated by sepsis and carries an up to 50% age-dependent mortality rate and severe long-term morbidity in survivors. Pure T-cell mediated IM-ADRs such as SJS/TEN are strongly associated with genetic variation in class I human leukocyte antigen (HLA) loci which has led to the development of successful preventive genetic screening such as HLA-B*15:02 screening in the prevention of carbamazepine SJS/TEN. Current information on key class I risk HLA alleles specifically associated with nevirapine SJS/TEN across diverse populations in Africa and Asia is lacking. A secondary unanswered question central to the mechanism of HLA-associated IM-ADRs is why hypersensitivity generally occurs in only a small proportion of those carrying an HLA-risk allele. We hypothesize that the immunophenotypic characterization and T-cell receptor (TCR) specificities of the drug-specific T cells in hypersensitive patients will help answer this question. To facilitate defining the specific immunopathogeneses of IM-ADRs, our research group has established global collaborations that have led to accrual of one of the largest international biobanks of cryopreserved PBMCs, blister fluid and skin biopsies from patients with severe T-cell-mediated ADRs at various time points from their acute reaction. We have recently identified HLA-C*04:01 as a significant class I risk allele in South African HIV+ patients with nevirapine SJS/TEN, and we have immunophenotyped the nevirapine specific pathogenic CD8+ T cells. These nevirapine-responsive cells were characterized based on their cytokine outputs, cell surface and activation markers (CD137+/ CD69+) using ELISpot assays, intracellular cytokine staining and flow cytometry following *ex-vivo* stimulation with nevirapine. To identify the TCR specificities of the T-cell repertoire in patients with HLA-C*04:01+ nevirapine SJS/TEN, single cell TCR sequencing for paired T-cell receptor alpha and beta genes and phenotypic markers will be performed to compare activated and non-activated populations of T cells in the presence and absence of drug. Candidate TCRs will be confirmed with a Jurkat TCR reporter assay. Our work leverages clinical and immunologic approaches to define the immunopathogenesis of nevirapine SJS/TEN. It is expected that these approaches will ultimately lead to translational outputs such as a sensitive predictive assay to identify the dominant cross-reactive TCRs in the peripheral blood of HLA-C*04:01+ patients who are destined to develop nevirapine SJS/TEN.

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Microscale Engineering of the Tumor Microenvironment for Therapeutic Targeting of Tumor-associated Macrophages in Prostate Cancer.

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The tumor microenvironment (TME) plays integral roles in prostate cancer progression and represents a high value therapeutic target. The development of effective TME-targeted therapies is limited by current technologies which are insufficient to replicate or analyze this complex environment. To address these challenges, we have developed a microfluidic cell culture platform known as STACKS, which permits co-culture of up to 6 patient-derived cell populations as well as compartmentalized, multiplexed analysis of gene expression, cell signaling, and matrix remodeling. We have focused on investigation of tumor-associated macrophages (TAMs), which are traditionally classified as M2 (tumor-supportive) or M1 (tumor-destructive) and are high value therapeutic targets with roles in prostate cancer growth, metastasis, survival, and therapeutic resistance. **Methods:** Cell line (THP-1) and patient-derived monocytes were differentiated into macrophages within the STACKs device and polarized to either a M1 or M2 state or left unpolarized. Unpolarized macrophages were cultured with androgen dependent (LNCaP) and independent (DU145,C4-2B) prostate tumor lines and cancer-associated fibroblasts (CAF) derived from patient biopsies. Cells were cultured on 2D surfaces as well as within 3D matrix environments. Individual cell populations were isolated and analyzed for RNA, protein, and secretory factor expression. **Results:** We report that M2 (CCL18, MRC1, IL-10) as well as M1 (CXCL10, CXCL11) associated genes in patient-derived macrophages were regulated by paracrine as well as mechanical stimuli in culture. In unpolarized macrophages, expression of MRC1 and IL-10 was upregulated by culture on hydrophilic surfaces and expression of all M2 genes was upregulated through culture in a 3D collagen I matrix environment. Additionally, culture of primary macrophages within 3D collagen I matrix in the

presence of interferon gamma and IL-4 synergistically upregulated M1 and M2 gene expression respectively. In co-culture experiments, while IL-10 expression was higher in THP-1s cultured with LNCaPs than with C4-2Bs on a 2D surface, the opposite was true when THP-1s were cultured within a 3D collagen I matrix. **Conclusions:** Within a microscale co-culture environment, we have demonstrated that macrophage gene expression is regulated both independently and synergistically by paracrine and mechanical factors. Expression of certain genes, such as IL-10, was strongly dependent on the integration of multiple TME signals, such as paracrine tumor factors and cell-matrix interactions. Our findings highlight the complexity of macrophage polarization within the TME and suggest that there is overlap between the intracellular pathways involved in paracrine and mechanical regulation of gene expression. These common pathways represent new therapeutic targets for prostate cancer treatment. We will continue to build on this data through 2D and 3D multi-culture of macrophages with the tumor, stromal, and immune cells to target pathways involved in TAM polarization, tumor promotion, and therapeutic resistance.

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Intratumor Heterogeneity Predicting Clinical Outcomes of EGFR Targeting in Metastatic Colorectal Cancer

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Frontiers in metastatic colorectal cancer (mCRC) include new applications of targeted therapeutics. Cetuximab has demonstrated enhanced response rates and survival benefit even in late-line settings. Unfortunately, challenges remain in identifying patients that benefit from these agents with current markers of resistance including *KRAS* and *NRAS* mutations. Tumor heterogeneity has been proposed as a mechanism of resistance though it clinical application remains a formable challenge. Non-invasive characterization of tissue structures could reveal tumor heterogeneity and may predict response to therapy. Using standard of care non-contrast CT imaging, texture analysis may expand features that predict intratumor heterogeneity. Previous work has shown that skewness (asymmetry of the pixel histogram) was correlated with *KRAS* wild-type status. We hypothesized that texture analyses including skewness would enhance the ability to predict benefit of cetuximab in the late-line setting. **Methods**: A training cohort of patients with mCRC who received cetuximab in the late-line setting were identified. CT texture analysis was performed on the largest index lesion prior to initiation of cetuximab. The patient population included a diverse background including multiple courses of prior chemotherapy and metastatic sites including hepatic, pulmonary, omental, and pleural lesions. **Results**: For patients with tumor >20mm similar correlates were observed with increasing skewness associating with enhanced progression free survival (PFS, R=0.51). In patients with tumor R=-0.56). Further analysis revealed inverse relationships for entropy (irregularity or complexity of pixel intensity, R=-0.57) and mean of positive pixels (average value of pixels >0, R=-0.66). **Conclusions**: This study provides the first clinical correlation to link pre-treatment tumor characteristics with targeted therapeutic response using CT texture analysis in mCRC. Ongoing work will expand this analysis in larger patient cohorts, examine inter-tumor variation in individual patients, and track texture analysis over the treatment course of targeted therapy. CT texture analysis holds great promise as a platform for non-invasive prediction of treatment response warranting further investigation.

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Evaluation of the Combinational Therapy Cyclosporine A and Phenelzine on Protection of Synaptic Mitochondrial Respiratory Function Following Severe Controlled Cortical Impact Injury in Rats

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Objective: To evaluate the ability of the combination, CsA and PZ, to additively or synergistically improve synaptic mitochondrial respiration following experimental TBI in comparison to either agent alone. **Background:** Traumatic brain injury (TBI) represents a significant health crisis in the United States and there are currently over five million people living with a TBI related disability. However, acute treatment of TBI remains supportive and there are no FDA-approved pharmacotherapies available to prevent the devastating neurologic consequences of TBI. Following TBI, mitochondria buffer increases in intracellular calcium in an attempt to maintain homeostasis, however, increases in intra-mitochondrial calcium lead to generation of reactive oxygen and nitrogen species (ROS/RNS), induction of lipid peroxidation (LP), and formation of the LP-derived aldehydes, 4-HNE and acrolein. 4-HNE and acrolein covalently bind mitochondrial proteins, exacerbating production of ROS/RNS, mitochondrial dysfunction, and energy impairment. Eventually, mitochondrial dysfunction leads to opening of the mitochondrial transition pore (mPTP), extrusion of calcium back into the cytosol, activation of calpain, cytoskeletal degeneration, neuronal death, and neurologic impairment. Mitochondria are heterogeneous, consisting of synaptic and non-synaptic mitochondria. Synaptic mitochondria are purely neuronally-derived and more susceptible to injury compared to non-synaptic mitochondria. Synaptic mitochondria are considered essential for proper neurotransmission and synaptic plasticity, and their dysfunction has been implicated in neurodegeneration. Therefore, synaptic mitochondria are promising therapeutic targets for prevention of cellular death and dysfunction following TBI. Individual administration of cyclosporine A (CsA), an immunosuppressant with the ability to inhibit mPTP, or phenelzine (PZ), an antidepressant with aldehyde scavenging properties, has been shown to partially attenuate mitochondrial respiratory function following experimental TBI. However, the combinational effects of the partially neuroprotective agents, CsA and PZ, has not been previously evaluated. **Methods:** Male Sprague-Dawley rats received a severe unilateral controlled cortical impact injury (CCI). Sham animals underwent craniotomy, but were not exposed to impact. Immediately following injury, mini-osmotic pumps were implanted containing CsA (10mg/kg/day in cremophor/ethanol/saline) or vehicle. Fifteen minutes following injury rats received an intraperitoneal 20mg/kg loading dose of CsA, a subcutaneous 10mg/kg dose of PZ in saline, and/or vehicle(s). A follow-up 5mg/kg subcutaneous dose of PZ or vehicle was given at 12h. At 24h rats were euthanized, purified synaptic mitochondria were isolated, and states of respiration were measured using a Clark-type oxygen electrode. **Results & Conclusion:** CsA protects synaptic mitochondrial respiration 24h following severe CCI. The ability of PZ and the combination of CsA + PZ to protect synaptic mitochondrial respiration 24h following severe CCI is evaluated in comparison to individual CsA neuroprotection.

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An Automated Microfluidic Assay for the Detection of Human Papillomavirus-associated Oropharyngeal Cancer Biomarker in Serum using Photonic Crystal Enhanced Fluorescence

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This work demonstrates the detection of cancer biomarkers in serum using a microfluidic system to automatically control the flow of reagents in fluorescence-linked immunosorbent assays (FLISAs), and a photonic crystal (PC) enhanced fluorescence (PCEF) detection system to scan completed assays. This combined platform was applied to the detection of anti-E7 antibody, a known biomarker for human papillomavirus (HPV)-associated oropharyngeal cancer (OPC), in spiked samples and clinical samples from 20 OPC patients and 20 healthy controls. The results were compared to concurrently-performed "gold standard" enzyme-linked immunosorbent assays (ELISAs). PCs are composed of alternating high and low refractive index dielectric materials arranged in a grating structure with sub-wavelength periods. In this setup, the PCs were designed to enhance the fluorescence signal of AlexaFluor 647 via "enhanced excitation" and "enhanced extraction" mechanisms. Detection and control proteins were printed in a microarray format onto the surface of 2 x 8 mm^2 PCs using a non-contact printer. The PCs were incorporated into acrylic-based microfluidic cartridges, into which 10 µL of serum sample was manually pipetted. All subsequent flow of reagents was automated through a micro-controlled 3-way valve and pressure-driven flow. Completed assays were removed from the microfluidic system and scanned on a custom-designed PCEF line-scanning system. While the PCEF results have ~70% agreement with ELISA, several discrepancies result in a relatively high rate of "false positives" which necessitate further clinical exploration. Overall, the experimental flow involving the microfluidic assay platform and PCEF-based detection is largely automated and has significant potential for multiplexing and can be adapted for the rapid, multiplexed detection of arrays of disease biomarkers, including other cancers and infectious diseases.

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Hypoxia Before Global Ischemia Drives Increased Apoptotic Neuronal Death

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Asphyxial cardiac arrest (ACA) is associated with greater brain injury than ventricular fibrillation (VF) cardiac arrest even with the same no flow or ischemia period yet the etiology remains unclear. Understanding the mechanistic explanation for why hypoxia preceding ischemia worsens neurologic injury may provide new targeted therapeutic opportunities. Hypoxic perfusion immediately preceding global ischemia (ACA) induces different pathways of cell death compared to abrupt ischemia (VF). **Methods:** E18 rat cortical neurons were prepared and underwent simulated ischemia by oxygen-glucose deprivation (OGD) on DIV10-12. We modeled brief ischemia only (45 m OGD; OGD_{45}), VF's abrupt ischemia/hypoglycemia (60 m OGD; OGD_{60}) or ACA's hypoxia preceding brief ischemia (15 m hypoxia + 45 m OGD; $H_{15}OGD_{45}$). Treatment media used was either OGD media (HBSS 0% O_2, no added glucose) or hypoxia media (HBSS 1.2% O_2, 25mM glucose). Caspase-3 and calpain activation were assessed by immunoblotting 24h cell lysates for αII-spectrin breakdown products. Annexin V and PI positivity were assessed by flow cytometry at 24h of reperfusion and neuronal viability by LDH assay at 6, 24, 48 and 72h. The biochemical basis of the observed differences was assessed via quantification of reduced glutathione (GSH) levels. **Results:** Annexin V binding and overall neuronal cell death, measured by LDH release, was minimal following OGD_{45} at all timepoints. $H_{15}OGD_{45}$ resulted in 33% increased Annexin V binding (p=0.011) and greater 72h neuronal death compared to OGD_{60}. The temporal patterning of treatment was found to be a significant factor affecting the evolution of cell death, in addition to overall duration of the insult. Cells were protected against OGD_{60} when preconditioned with a 15m brief OGD (60m reperfusion between OGD treatments). $H_{15}OGD_{45}$ resulted in 64% more caspase 3 activation and caspase-dependent αII-spectrin cleavage than OGD_{60} (p=0.02), and both conditions caused significant calpain activation ($p15OGD_{45}$(p=0.005) but not OGD_{60} compared to normoxia control. **Conclusions:** Our data suggests, hypoxia immediately preceding otherwise non-injurious ischemia substantially worsens injury through neuronal apoptosis. These finding correlate with clinical observations and serve to provide a foundation for future exploration of the mechanistic differences between VF and ACA.

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Variation in Short Tandem Repeats of *Vasopressin Receptor 1a* are Associated with Violent Offending

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Vasopressin is an ancient multifunctional neuropeptide whose many effects are mediated by the varied distribution of its three distinct receptors. Short Tandem Repeat (STR) polymorphisms in the flanking region of the *Vasopressin Receptor 1a* (*AVPR1a*) gene have attracted interest in social neuroscience due to their association with altered *AVPR1a* expression in the brain and with a number of social behaviors including pair-bonding, altruism, and aggression. The RS1 STR, a tetranucleotide repeat situated upstream from the transcription start site with 9 reported alleles, has been linked to autism, the 310 base pair allele carrying increased risk. We studied the distribution of RS1 alleles and the relationship of the RS1 310 risk allele to violent crime in subjects with a personal and family history of violent crime, and in healthy controls. **Methods:** The sample consisted of 288 Finnish males: 91 were incarcerated for violent crimes, 67 were relatives of the incarcerated criminals with no history of violent crime, 103 were controls, 2 were relatives of the controls, and 25 were relatives of the incarcerated criminals who were also incarcerated for violent crimes. The RS1 STR was genotyped by size with an ABI 3730 Capillary Sequencer and using primers that equivalently amplified the various RS1 alleles. The allele frequency of the 310 risk allele was compared

between cases (incarcerated criminals with relatives who were also incarcerated criminals, n=25) and controls (n=103). **Results:** 7 RS1 alleles were detected with lengths ranging from 306 to 331 base pairs. The 310 allele was the most common (allele frequency (AF) = 0.33), followed by the 315 allele (AF = 0.19), the 306 allele (AF=0.19), the 323 allele (0.13), the 319 allele (0.11), the 331 allele (0.05), and the 327 allele (0.003). The 310 allele was associated with a decreased tendency towards violent crime (AF in controls = 0.58, AF in cases = 0.32; OR = 0.33, p=0.0009). **Conclusions:** Variation in the number of repeats of the RS1 allele, particularly the presence or absence of the 310 base pair allele, may contribute to heritable aggression. This allelic effect may be mediated through altered expression of the *AVPR1a* gene in different regions of the brain, a possibility that is now being evaluated.

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Dissecting the Roles of Different Complement Pathways in Secondary Injury after Brain Trauma Using Translational Injury-Site Targeted Inhibitors

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After initial mechanical insult, traumatic brain injury (TBI) is characterized by a dynamic process of secondary injury that involves chronic neuroinflammation, which is a lead determinant of long-term cognitive and motor recovery. Complement activation is a major component of the inflammatory cascade, and there is increasing evidence that complement plays a role in propagating injury after TBI. The aim of this study is to investigate, using injury-site targeted complement inhibitors, the roles of different complement pathways in promoting injury after TBI while assessing the therapeutic efficacy of pathway-specific complement inhibitors. The complement inhibitors used in these studies are fusion proteins of complement receptor 2 (CR2) linked to one of three complement inhibitors: Crry (inhibits all complement pathways), fH (inhibits the alternative pathway), or CD59 (inhibits the terminal pathway). CR2 specifically binds to the sites of complement activation (C3d deposition), thus targeting the complement inhibitor to the site of brain injury after murine moderate controlled cortical impact (CCI). A single dose of either CR2fH (16mg/kg) or CR2Crry (10mg/kg) administered intravenously 1 h after CCI significantly reduced acute laterality on corner task ($p < 0.05$), significantly improved motor function on the ladder task, and significantly improved cognitive performance on Barnes maze, as assessed one month after insult ($p < 0.05$). Acute pan-inhibition of complement activation (CR2Crry) or of the alternative pathway (CR2fH) significantly reduced expansion of primary injury and associated astrogliosis, reduced chronic deposition of C3d in the brain, and inhibited microglial activation while maintaining neuro-regenerative mechanisms including neurogenesis and neuronal migration. Treatment with CR2CD59 (6mg/kg, molar equivalent) resulted in similar acute improvement in forelimb laterality, but did not result in improved cognitive and motor performance chronically compared to vehicle controls. Further, CR2CD59 did not result in a robust decrease in complement and microglia activation chronically. These findings indicate that the terminal complement pathway contributes to acute neuronal loss after TBI, but that chronic neuroinflammation and propagation of secondary injury is predominantly mediated by earlier complement activation products (C3 opsonins and anaphylatoxins), production of which is amplified by the alternative pathway. Finally, site-targeted inhibition of the alternative pathway using CR2fH has a strong translational potential for TBI therapy given that a humanized version (TT30) has been found to be safe and non-immunogenic in humans.

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Eosinophils Regulate Airway Substance P Levels

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Asthma is a heterogeneous disease with airway inflammation that may be eosinophilic or neutrophilic. Inflammation may contribute to the airway nerve dysfunction that is characteristic of asthma, causing bronchoconstriction and airway hyperreactivity. Airway sensory neurons cause bronchoconstriction both indirectly, by providing the afferent limb of the parasympathetic reflex arc and directly, via release of substance P, a neuropeptide that evokes cough, bronchoconstriction, inflammation, and vascular leakage. Thus we sought to define the effects of eosinophils on nerve substance P expression and its contribution to airway reactivity. **Methods**: Eosinophil-deficient ((-)Eos) and wild-type (WT) mice were sensitized to house dust mite antigen (HDM, 50 µg intranasal) on days 0 and 1 and challenged (25 µg intranasal) on days 14-17. Control animals received intranasal saline alone. On day 18, animals were mechanically ventilated and the increase in airway resistance in response to inhaled serotonin was measured. In some animals, substance P receptors were blocked with a neurokinin-1 receptor antagonist (CP99994 20 µg inhaled). Substance P breakdown by neutral endopeptidase was inhibited with phosphoramidon (2.5 mg/kg i.v.). Bronchoalveolar lavage substance P was measured by ELISA. Whole mount tracheas were immunofluorescently labeled using antibodies to the pan-neuronal marker PGP9.5 and to substance P. Tracheas were optically cleared and confocal images were used to construct three-dimensional computer models of airway nerves to calculate substance P positive nerve volume. **Results**: HDM exposure increased eosinophils in airway lavage fluid in WT mice ($p < 0.0001$) compared with saline controls. In contrast, in (-)Eos mice, HDM exposure increased lavage neutrophils ($p=0.02$). HDM exposure potentiated serotonin-induced bronchoconstriction in WT mice ($p < 0.0001$), but not in (-)Eos mice. Blocking substance P receptors decreased airway reactivity in WT HDM-treated mice ($p=0.0477$). HDM treatment increased both neuronal ($p=0.0004$) and bronchoalveolar lavage ($p < 0.0001$) substance P content, irrespective of eosinophilic or neutrophilic inflammation. Inhibiting neutral endopeptidase, and thus substance P breakdown, did not further increase airway reactivity in WT HDM-treated mice, but did increase airway reactivity in (-)Eos HDM-treated mice ($p=0.03$). **Conclusion**: Eosinophils increase airway reactivity in asthma. Both eosinophilic and neutrophilic inflammation increased airway substance P levels, but substance P contributed to airway reactivity only in WT mice due to impaired breakdown by neutral endopeptidase. Eosinophil-dependent changes in airway neurotransmitters may contribute to nerve dysfunction in asthmatic patients.

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Src-family Kinases and Src-activating Signaling Molecule Regulate Corneal Epithelial Wound Healing

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Corneal wounds are a significant global concern that can lead to scarring and blindness. Studies suggest activation of epidermal growth factor receptor (EGFR) and Src-family kinases(SFKs) may play a role in corneal epithelial wound healing. In skin keratinocytes, Src-activating signaling molecule(Srcasm) has been shown to regulate EGFR and SFK signaling. Srcasm is a SFK substrate downstream of EGFR that can preferentially activate Fyn and Src, and has been shown to downregulate activated SFKs and EGFR in a lysosomal-dependent manner. To further understand the role of SFK and Srcasm in corneal epithelial cells, wound healing assays and Western blot analysis were performed using mouse corneal epithelial cells (MCEC) derived from Srcasm and Fyn null mice, as well as immortalized and primary human corneal epithelial cells in the presence and absence of a tyrosine kinase inhibitor. **Methods:** MCEC cultures were established and subsequently wounded from: (1) Srcasm wildtype (WT); (2) Srcasm heterozygote (*Srcasm*$^{+/-}$); (3) Srcasm knockout (*Srcasm*$^{-/-}$); and (4) Fyn knockout (*Fyn*$^{-/-}$) mice. Monolayers derived from 2.040 pRSV-T ATCC immortalized corneal epithelial cells (2.040) and primary human corneal epithelial cells (HCEC) were also established; incubated with dasatinib, a small molecule tyrosine inhibitor, or vehicle at various concentrations; and subsequently wounded. Wound healing studies were performed and analyzed using a live cell imaging system and Slidebook software. Parallel cultures were wounded for Western blot analysis to measure activated EGFR, SFK, and Srcasm levels. **Results:** Wound healing studies showed accelerated wound healing by corneal epithelial cells from *Srcasm*$^{-/-}$ mice and slower wound healing from *Fyn*$^{-/-}$ mice compared to WT. HCEC and 2.040 cells incubated with dasatinib showed dose dependent inhibition of wound healing, while control cells filled nearly the entire wounded area after 24 hours. Wound healing assay results correlated with levels of activated EGFR and SFK, but inversely with Srcasm levels. **Conclusion:** Wounded corneal epithelial cells demonstrate enhanced wound healing activity with loss of Srcasm, which is associated with increased activation of EGFR and SFK seen on Western blot analysis. Diminished wound healing activity, on the other hand, is observed with cells derived from Fyn null mice or SFK inhibition. Results from this study suggest a critical role for EGFR and SFK activation in corneal epithelial wound healing with Srcasm modulating EGF-dependent signaling through activation of SFK. Studies to determine how Srcasm regulates activation of EGFR and SFK in corneal epithelial cells should be conducted.

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Multimodal Neural Correlates of Cognitive Control in the Human Connectome Project

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Cognitive control refers to the set of cognitive functions that are involved in coordinated, purposeful decision making processes guided by internal goals, states, and rules. There are numerous studies of cognitive control in the neuroimaging literature. However, many studies focus on solely a single modality of imaging data, such as task imaging, resting state imaging, or structural imaging, which provides limited views of neural functioning. Recent methodological developments have yielded frameworks that enable simultaneous analysis of multiple imaging modalities. These approaches capitalize on the strengths of individual modalities and provide windows into neural functions at multiple levels of brain organization. Thus, we performed multimodal analyses using large participant groups (cohort1 n=193; cohort2 n=149) from the Human Connectome Project to determine neural correlates of cognitive control. Measures of cortical thickness (sMRI), resting-state functional connectivity (rsfcMRI), and two functional tasks (tfMRI: working memory [WM]; relational processing [REL]) were analyzed using multiset canonical correlation analysis with joint independent components analysis (mCCA+jICA). mCCA+jICA yielded a set of independent components (ICs), each of which contained results for all four modalities and subject-specific weights on these ICs. Cohort1 was analyzed and subject-specific weights were correlated with a composite cognitive control performance score. All four modalities in one of Cohort1's ICs significantly correlated with cognitive control after FDR correction (sMRI: r=0.182,p=0.011; rsfcMRI r=0.442,p < 0.000; REL-tfMRI r=0.224,p=0.002; WM-tfMRI r=0.277,p < 0.000). Visual analysis of this IC's modality maps identified: sMRI contributions from the insula, temporal poles, and cingulate; REL-tfMRI contributions from visual cortex, superior and inferior parietal cortex, inferior temporal cortex, left supramarginal gryus, left precentral sulcus, and right rostral middle frontal cortex; WM-tfMRI contributions from visual cortex, left middlefrontal gyrus, bilarteral supramarginal gyrus, and right superior parietal gyrus; and rsfcMRI contributions from strong within-network connectivity as well as anticorrelations between several task-positive networks and the default mode network. We also replicated findings using two methods. First, we used the results from cohort1 to predict cohort2. The corresponding correlations in cohort2 were significant for three of the four modalities (rsfcMRI r=0.225,p=0.006; REL-tfMRI r=0.398,p < 0.000; WM-tfMRI r=0.213,p=0.009). Second, we independently analyzed data from cohort2 using mCCA+jICA. We used an automated similarity-matching algorithm to pair ICs across cohorts. This identified an IC in cohort2 that was most similar to cohort1's IC and was also associated with cognitive control. All four modalities for this IC in cohort2 significantly correlated with cognitive control after FDR correction (sMRI r=0.254,p=0.002; rsfcMRI r=0.234,p=0.004; REL-tfMRI r=0.408,p < 0.000; WM-tfMRI r=0.437,p < 0.000). This set of findings underscores the utility of multimodal analyses and suggests utility in their application to other areas in neuroscience and psychopathology.

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Parabiosis Reveals Renal Resident Leukocytes in Quiescence and Acute Kidney Injury

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Inflammation drives damage and promotes tissue regeneration in acute kidney injury (AKI), but the origin of inflammatory cells found in renal tissue (infiltrative versus tissue-resident) has remained elusive. In this study, we developed a novel model of AKI in parabiosis chimeras to study exchange of inflammatory cells with the circulation during acute and subacute inflammation. **Objectives:** We investigated the extent of exchange of leukocyte populations between the kidney and the peripheral circulation during quiescence and following acute injury using the parabiosis model. Overall, our goal was to discern which renal leukocyte populations are tissue-resident, which we define as demonstrating no overlap of the 95% confidence interval for chimerism levels, and how this may change in the setting of inflammation. **Methods:** Parabiosis was established between C57BL/6J adult mice congenic for the CD45 allotypes, allowing cells from each individual to be identifiable. After 28 days, parabiotic chimeras were subject to 30 minutes of renal ischemia followed by reperfusion for 24 or 72 hours before analysis. Control groups were subjected to a sham procedure. Kidney, peripheral blood, and secondary lymphoid organs were analyzed by multicolor flow cytometry. **Results:** After 28 days of parabiosis, we observed that chimerism in the peripheral blood (37.5% of $CD45^+$ cells; 95% CI, 30.9 to 44.2%) and spleen (32.9% of $CD45^+$ cells; 95% CI, 29.4 to 36.4%; Mann-Whitney, $p = 0.31$, n = 6 pairs) was not different. Further, we observed similar but lower chimerism among renal leukocytes known to have high turnover (neutrophils 24.2%; 95% CI, 14.2 to 34.2%; $p = 0.015$ vs blood). In contrast, compared with blood chimerism, $F4/80^{Hi}CD11b^{Low}CX3CR1^{Hi}CD11c^+$ macrophages, $CD3^+CD4^-CD8^-$ T lymphocytes, and $NK1.1^+CD3^+$ NKT cells appeared to be resident, with chimerism equal to 2.4% (95% CI, 1.0 to 3.8%; $p = 0.002$), 2.3% (95% CI, 0.6 to 4.1%; $p = 0.02$), and 2.3% (95% CI, 0.7 to 3.9%; $p = 0.002$), respectively in uninjured kidneys. In injured kidneys, a trend toward chimeric $CD45.1^+$ leukocyte infiltration was observed relative to sham control (6.6×10^5 1.1×10^4 vs 1.8×10^5 8.4×10^4 cells/g tissue, $p = 0.10$, n = 3 pairs, 24h after injury). However, no change was observed in absolute numbers of chimeric $F4/80^{Hi}CD11b^{Low}CX3CR1^{Hi}CD11c^+$ macrophages, indicating bone marrow precursors from the peripheral blood do not supplement expansion of this population even in the setting of acute inflammation. **Conclusions:** We found specific renal leukocyte populations that exhibit low or no exchange with the peripheral blood, indicating they are either long-lived or undergo self-renewal *in situ*. Kidney resident macrophages do not appear to be supplemented by infiltrating cells during acute inflammation. These findings may be important in targeting inflammation after AKI with small molecule drugs or development of cell-based therapeutics.

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Role of the Heterogeneous Auditory Thalamus Neurons in Auditory Conditioning

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Understanding the neurological mechanisms underlying emotional learning is of tremendous importance given that the process is implicated in addiction, autism, and anxiety disorders. The amygdala has long been understood as an essential part in processing both fearful and rewarding environmental stimuli, where auditory memories are reputedly acquired and stored. This widely accepted amygdala model holds that the auditory conditioned stimulus (CS) and the unconditioned stimulus (US) converge in the lateral nucleus of the amygdala (LA), and are projected from the medial geniculate nucleus (MGN) and the adjacent posterior intralaminar nucleus (PIN), which are understood merely as sensory relays. However, recent studies have implicated the MGN/PIN with the LA using synaptic and molecular approaches. Yet, it is unclear what role the auditory thalamus neurons play in auditory conditioning. Here, we used an in-vivo electrophysiological approach to record neural activity within the MGN/PIN during Pavlovian auditory conditioning in mice. Mice were conditioned in sound-proof boxes that contained a modular test cage assembled with a sucrose delivery port, a speaker, and a house light placed under the sucrose port. They learned to associate three different tones (CS) with either a sucrose reward, foot shock, or neither. These animals were able to discriminate between the CS+ (tone associated with reward) and the CS- (neutral tone), measured by the difference of latency to sucrose port entry between the two stimuli ($p < .001$). Neurologically, we found MGN/PIN neurons that responded to both CS+ (reward and foot-shock tones), but only after learning. We also found neurons that responded to the stimulus valence and those that predicted behavior. Overall, these findings demonstrated that auditory thalamus neurons are heterogeneous and play a more significant role in Pavlovian conditioning than previously thought.

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Combination Therapy Increases Lifespan and Improves Clinicobehavioral Performance in the Murine Model of Globoid Cell Leukodystrophy

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Globoid cell leukodystrophy (GLD, Krabbe Disease) is a rapidly progressing, pediatric lysosomal storage disease that results from the deficiency of galactosylceramidase (GALC). Toxic accumulation of galactosylsphingosine (psychosine, Psy), in *GALC-/-* oligodendrocytes and Schwann cells causes dysmyelinating CNS and PNS phenotypes. To date, no experimental single therapy in the murine GLD model (Twitcher, Twi) has extended the median Twi lifespan beyond 100 d. We previously showed that a mechanism-based combination therapy consisting of a second generation AAV vector (AAV2/5-GALC), bone marrow transplantation (BMT), and substrate reduction therapy (SRT) dramatically improved Twi lifespan to a median of

298 d (141-454 d). Although combination-treated Twi mice showed significant clinicobehavioral improvements, they were unable to perform the wirehang test at WT levels, suggesting incomplete disease correction. In this study, we show that gene delivery with the third-generation AAV2/9 vector acts synergistically with BMT and SRT to increase the Twi lifespan to a median of 346 d (157-541 d). This is accompanied by improvements in rotarod and wirehang performance. Eight of 16 combination-treated Twi mice stayed on the inverted wirehang for 60 s at 20 weeks of age. Three treated mice stayed on the wirehang and 14 treated mice stayed on the rotarod for the full 60 s until death. Importantly, of the sixteen Twi mice that received combination therapy, only two died from GLD. Fourteen of 16 treated Twi mice and 19 of 19 treated WT control mice died from AAV-induced HCC. The high penetrance of AAV-induced HCC makes it impossible to determine the efficacy of this triple-therapy regimen. However, it is likely that the median lifespan would have been significantly greater than 346 d if it were not for the HCC. This is supported by the observation that Twi mice treated with the double combination therapy of AAV2/9-GALC gene therapy and BMT have a median lifespan of 269 d, with some mice still alive at 450 days, while Twi mice treated with AAV2/5-GALC gene therapy and BMT have a median lifespan of only 123 d (91-283 d). Use of a vector with a weaker promoter will likely decrease the incidence of HCC while still providing therapeutic benefit.

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Upregulation of MicroRNA-150 in Aged Macrophages Promotes Age-related Macular Degeneration

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Age-related macular degeneration (AMD) is a leading cause of blindness in adults over 50 years of age in industrialized countries. We have reported that aged macrophages exhibit functional shifts in their activation status and lose their ability to maintain cholesterol homeostasis with both of these changes contributing to AMD pathogenesis. However, the role of microRNAs (miRs) in regulating these functional shifts in aged macrophages is unexplored. The goal of this study was to identify the miRs that coordinate the changes in gene expression networks that contribute to AMD pathogenesis. To identify candidate miRs that regulate macrophage aging, we performed a miR microarray, comparing thioglycolate-elicited peritoneal macrophages from 6-8-week-old mice and 18-month-old wild-type mice (C57BL/6J). After validation of expression changes by real-time PCR, we identified miR-150 as the candidate with the highest fold changes in aged macrophages with ~9-fold higher expression in old peritoneal macrophages and ~3-fold higher expression in old splenic and old bone marrow-derived macrophages. To validate these findings in human subjects, we quantified miR-150 levels in the peripheral blood mononuclear cells (PBMCs) of patients with early or advanced (wet) AMD and control subjects without AMD. Our multinomial logistic regression model showed that after controlling for age and gender, a ten-fold increase in PBMC miR-150 levels was associated with an odds ratio of 13.7 of having early AMD (95% CI: 2.4-78.3) and an odds ratio of 81.5 of having wet AMD (95% CI: 9.1-731.6). Next, to identify the gene networks regulated by miR-150, we performed RNA sequencing to compare the transcriptome of young macrophages transfected with synthetic miR-150 and that of young macrophages transfected with a non-targeting negative control. We applied cutoffs of |fold-change|≥1.2 and $p < .05$ and further filtered the list for only genes that were also identified in a previous microarray comparing aged and young macrophages (|fold-change|≥1.2; $p < .05$; FDR < .2). This strategy revealed 160 commonly dysregulated genes in both miR-150- versus negative control-transfected macrophages and old versus young macrophages. We performed functional analysis with MetaCore on this gene list for enrichment by pathway maps, process networks, and gene ontology processes. Of interest, aberrant lipid trafficking and metabolism in AMD pathogenesis was identified as the third most significant pathway map ($p=4.4\times10^{-5}$; FDR=5.5×10^{-3}), validating our hypothesis that miR-150 coordinates transcriptomic changes that are critical in AMD pathogenesis. Taken together, our results demonstrate that miR-150 is upregulated in AMD-promoting, aged macrophages and implicate miR-150 as pathogenic in AMD progression. Further studies will investigate the direct miR-150 targets that mediate these transcriptomic changes. Ultimately, the results of this study identify not only potential therapeutic targets for AMD that could be used to modulate the transition to the aged macrophage phenotype but also a potential biomarker for monitoring AMD progression.

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Genetic Determinants for Leisure Time Physical Activity in African and Caucasian Americans

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Leisure-time physical activity (LTPA) is a well-established modifiable factor that contributes substantially to cardio-metabolic health. However, whether and to what extent LTPA is affected by genetic factors remains unknown. **Methods:** To unveil the genetic basis of LTPA, we conducted a genome-wide analysis using 1000 Genomes Project imputed data from three major cohorts of women and men in the US, the Women's Health Initiative (n=11,865), the Jackson Heart Study (n=3,015) and the Framingham Heart Study (n=7,339). Specifically, in the primary analysis ethnicity-specific genetic signals were investigated comprehensively and in-depth for LTPA using GWAS-based meta-analysis. In addition, we conducted a candidate gene analysis of eight previously reported genes. To follow up on the findings, a series of secondary analyses were performed to further fine-map and functionally annotate the confirmed loci and explore the potential biological pathways. **Results:** In the primary analysis, two loci were identified in African Americans (AA), and one locus was identified in Caucasian Americans (CA). The lead single nucleotide polymorphisms (SNPs) from the loci identified for AA were rs116550874 (meta-analysis: z-score = -5.24, P = 1.63E-7) and rs3792874 (meta-analysis: z-score = 4.93, P= 8.33E-7); the lead SNP from the locus identified in CA was rs28524846 (meta-analysis: z-score = 4.83, P = 1.30E-6). In addition, we also found evidence for four previously reported loci (*GABRG3*, *CYP19A1*, *PAPSS2* and *CASR*). Further fine-mapping and functional annotation showed that the confirmed loci (novel and previously reported) may be involved in 1) the homeostatic drive coupled with the reward system for PA and 2) the development and regulation of capacities to perform PA. **Conclusions:** To our knowledge, our analysis is the first to

comprehensively investigate the genome-wide signals for human LTPA in multiple ethnicities. These findings support the notion that genetic predisposition plays a critical role in determining LTPA, of which the biological and clinical implications warrants further investigation.

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Network Identification, Interaction, and Variable Expression in Variant Idiopathic Pulmonary Fibrosis

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Idiopathic pulmonary fibrosis (IPF) is a complex disorder characterized by pulmonary fibrosis that leads to significant hypoxemic respiratory insufficiency and, ultimately, significant morbidity and mortality. Although there is currently no known etiology of IPF, familial clustering and association of IPF with systemic genetic syndromes suggest that genetics have a critical role in the development of IPF. We have previously shown that the *MUC5B* promoter variant (rs35705950) is strongly associated with sporadic and familial IPF but the exact mechanism is unknown. A previously published genome-wide association study identified 10 loci of susceptibility (encompassing 66 genes) involved in host defense, cell-cell adhesion, and DNA repair. We hypothesize that these genes are expressed and interact as a network in IPF. **Methods:** In this study, we identified the most promising network from the previously-identified loci by Ingenuity Pathway Analysis (figure 1). In this network, we selected four genes- AZGP1, OBFC1, DISP2, and the Androgen Receptor- to serve as representatives of this network and compared expression of these genes in healthy and IPF tissue by immunohistochemistry (IHC). **Results:** These four genes were notably down-regulated in diseased IPF tissue with both wild-type and heterozygous MUC5B variant as compared to healthy lung tissue. Surprisingly, the Androgen Receptor appeared to be strongly expressed in the *MUC5B* variant and in healthy tissue but lost expression in all other diseased tissue. **Conclusions:** This data suggests that there is an overall decreased expression of this network in IPF that is somehow altered by the *MUC5B* variant. Further studies of this network will be crucial to elucidate the mechanism of the *MUC5B* variant's effect on the development of IPF.

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Sustained Delivery of Growth Factor on Porous Sutures to Modulate the Proliferative Stage of Canine Flexor Tendon Repair

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Surgical repair of connective tissues such as tendons is challenging clinically, primarily due to failure of the injured site to adequately restore strength during the first few weeks following repair. Despite decades of improvements to suture materials, suture grasping methods, and rehabilitation techniques, as many as 48% of intrasynovial flexor tendon repairs result in repair-site elongation and catastrophic failure. Current surgical approaches attempt to hold tissues together throughout the first several weeks after repair, but do nothing to regulate the biology of healing. Systemic drug delivery is insufficient to promote healing in hypovascular tendon tissue. While tissue engineering approaches to modulate healing are promising, they typically substantially alter clinical techniques and are still far from clinical application. In this study, we delivered connective tissue growth factor (CTGF) coated onto porous sutures directly to the inside of the tendon tissue to promote the proliferative stage of tendon repair in a clinically-relevant, 14 day canine flexor tendon surgical model. Previous attempts to deliver biofactors on sutures were limited by bolus biofactor release and low loading capacity on solid sutures. Here, we modified the commercially available nylon sutures used in human flexor tendon repair (Supramid) to create micrometer-sized pores in the outer sheath without modifying the inner, load-bearing nylon fibers. The modified suture mechanical properties (e.g., strength, stiffness) were the same as unmodified Supramid sutures, as evaluated by single-strand mechanical tests and biomechanical tests of clinical-style, 8-stranded *ex vivo* repairs of canine flexor digitorum profundus tendons performed by an experienced hand surgeon. We loaded these porous sutures with CTGF in a heparin/fibrin-based delivery system (HBDS) that enabled sustained release over two weeks. Growth factor release kinetics performed *in vitro* demonstrated sustained release of 0.50 – 1.25 ng CTGF/cm suture/day over 14 days, corresponding to concentrations of 60-150 ng/mL released daily from suture within 3 mm tendon laceration site. To determine the effects of local CTGF delivery from porous sutures on the repair of intrasynovial flexor tendons in a clinically relevant, large animal *in vivo* model, we performed a series of flexor tendon repairs on 10 adult female canines in a paired fashion. Two flexor digitorum profundus tendon transections and repairs in Zone 2 were performed per animal, comparing porous sutures loaded with HBDS ± CTGF. After 14 days of controlled passive motion rehabilitation, the animals were euthanized and tendons were assessed by histology (n=3), gene expression and proteomics analyses (n=7) for markers of inflammation, cellular proliferation, extracellular matrix synthesis and remodeling. This work represents a novel, highly clinically translational approach to modify the biological healing response with almost no changes to the surgical operation, and only natural biological components. This biofactor delivery approach can be readily adapted to other surgical repairs.

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Chemotherapy-induced Kinome Reprogramming in Patient-derived Xenograft Models of Pancreatic Cancer.

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Pancreatic cancer is the 3rd leading cause of cancer deaths in the United States and its incidence is rising. Despite marginal improvements in chemotherapeutic regimens consisting of multiple cytotoxic agents, targeted therapies against molecular drivers of pancreatic cancer have all failed to meaningfully improve patient survival. Kinase inhibitors have proven to be effective therapies in other cancer types, and many potent and selective inhibitors already exist for kinases with poorly defined roles in pancreatic cancer. However, there have been relatively few studies of global kinase signaling in pancreatic cancer that have the potential to identify treatment-specific resistance mechanisms and promising rational combination therapies. This project will investigate the adaptive

response of the kinome to chemotherapies in pancreatic cancer. Patient-derived tumor xenografts (PDX) grown in mice were collected both before and after treatment with chemotherapeutic regimens, including 5-fluorouracil with folinic acid (5-FU), 5-FU with oxaliplatin (FOLFOX), and gemcitabine. Intracellular kinases were enriched in these tumor lysates using multiplexed kinase inhibitor beads (MIBs) and quantified using mass spectrometry, allowing for comparison of the activation state of the kinome before and after treatments in the same tumor. Compared to pre-treatment controls, PDX tumors treated with 5-FU and FOLFOX displayed higher expression of c-Jun N-terminal kinases 1/2 (JNK) and other mitogen-activated protein kinases (MAPKs) known to activate JNK. Surprisingly, the mechanistically similar chemotherapeutic agent gemcitabine did not induce the same increases in JNK expression or preferential activation of MAPKs upstream of JNK. JNK1 and JNK2 are known to translocate to the nucleus to activate transcription factors that regulate critical stress response, cell cycle, and apoptosis pathways, but their roles in mediating drug resistance are not well understood. In PDX cell lines, we have observed that pharmacological JNK inhibition strongly synergizes with 5-FU and FOLFOX to inhibit cell growth, and are planning a study to assess the in vivo ability of JNK inhibition to inhibit tumor growth in combination with chemotherapies. We are investigating the mechanism that leads to activation of JNK following chemotherapy and observed synergy by creating genetically altered cell lines, including CRISPR-Cas9 JNK1/JNK2 knockout lines and lentiviral-mediated overexpression of JNK1/2 constructs with and without mutations predicted to disrupt the ability of JNK to be activated or bind to drug. This study has the potential to uncover specific signaling pathways involved in the adaptive response to chemotherapies, and to inform future work into JNK as a target in pancreatic cancer. Moreover, this experimental strategy has broad applicability to other treatments and cancer types, allowing this work to add to our growing understanding of how to most effectively identify and test novel drug targets.

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Neuroanatomical Analysis of a Conditional Knockin Mutant *PHOX2B* Mouse Model

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Central Congenital Hypoventilation Syndrome (CCHS) is a rare disorder characterized by autonomic dysfunction, including abnormal respiration. Symptoms of CCHS include the inability to establish a regular respiratory rhythm, inadequate responses to hypoxemia and hypercapnia, heart rate abnormalities, and impaired bowel function. Mutations in paired-like homeobox 2b (*PHOX2B*) are known to be causative for CCHS. We previously demonstrated that mice harboring an eight-nucleotide deletion in *PHOX2B* (d8-*PHOX2B*) displayed diffuse hindbrain pathology and perinatal lethality due to lack of spontaneous respiration. In this study, we are further exploring which brainstem circuits are required at birth for proper respiratory function and whether specific circuits are lost or insufficiently matured in mice expressing d8-*PHOX2B*. **Methods**: We are crossing several Cre driver mice with d8-*PHOX2B* mice to selectively express d8-*PHOX2B* in hindbrain neural nuclei known to be affected by mutant *PHOX2B* expression. Histological examination of brainstems from these mice along with unbiased quantification will allow us to determine how the morphology and number of cells within these neural populations is altered. **Preliminary results**: Atoh1-cre; d8-*PHOX2B* mice were generated and assessed for perinatal viability, as Atoh1 is a transcription factor known to be involved in the development of medullary respiratory centers. At birth Atoh1-cre; d8-*PHOX2B* pups appeared healthy and displayed spontaneous respiration. Histological examination of hindbrains from these pups revealed no neuropathology, with evident intact locus coeruleus, facial nucleus, and retrotrapezoid nucleus. These preliminary results suggest that the expression of Phox2b in the Atoh1 lineage is not required for normal hindbrain development and respiration at birth.

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Epigenetic Modification in Cooperation with Progesterone Receptor Activity Drives the Dysregulation of RANKL Gene in Uterine Leiomyoma

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Uterine leiomyoma (LM), the most common benign tumor in women, causes excessive bleeding, anemia, recurrent pregnancy loss, and may mimic or mask malignant tumors. Although LM growth requires ovarian steroid hormones, the underlying mechanisms remain unclear. Recently, we have demonstrated that receptor activator of nuclear factor kappa-B ligand (RANKL) was significantly dysregulated in LM tissue compared with adjacent normal myometrial (MM) tissue and played a tumor-promoting role by inducing proliferation and inhibiting apoptosis in LM cells. Here, we aim to investigate the mechanisms by which RANKL is regulated in LM versus MM tissue.

Using freshly collected LM and MM tissues from premenopausal women undergoing hysterectomies, we demonstrated that in vivo RANKL expression was dramatically higher ($N=17$, $p < 0.001$) and in vitro it was more robustly induced by progesterone (P) agonist R5020 in LM compare with MM. Despite that PR expression is similar between LM and MM, PR binding towards the RANKL proximal promoter and distal enhancer (75kb upstream of RANKL transcription start site) regions was significantly higher in LM vs MM ($N=10$, $P < 0.01$). Regression analysis indicated that recruitment of PR to the enhancer region of RANKL gene was significantly and positively correlated with its mRNA level, suggesting that RANKL expression is influenced by PR binding activity. Because DNA methylation is a key regulator of transcription factor binding (eg. PR), we used MethylCap-Seq to evaluate the genome-wide methylation status of LM and MM. The distal enhancer region of RANKL gene was found to be hypermethylated in MM tissue ($N=3$, $p < 0.05$), which reduced PR binding to this region. Treatment with DNA methylation inhibitor 5'-aza reduced DNA methylation level of the enhancer region and stimulated RANKL expression in both MM and LM explants, but the change in MM is much more dramatic ($N=5$). In addition, ChIP assay revealed that H3K27Ac marking in the enhancer region and H3K4me3 marking in RANKL promoter region are much more enriched in LM tissue ($N=5$).

These findings suggest that DNA methylation, histone modification, and PR signaling together constitute a complicated regulatory network to control RANKL expression in LM tissue. Our studies represent a key step towards the better understanding of mechanisms underlying the pathogenesis of LM and indicate the necessity for personalized disease therapeutics.

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Microscopic Image Guidance: Real-time Radiofrequency Ablation Monitoring for Barrett's Esophagus

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The term "image-guided therapy" has traditionally been confined to predominantly *macroscopic* imaging modalities, such as computed tomography and ultrasound imaging that offer spatial resolution on the order of millimeters, for guiding therapeutic interventions. Here, the notion of *microscopic image guidance* is introduced. The overall goal of this work is to develop a framework for real-time guidance and monitoring of thermal therapy in epithelial lesions that seamlessly integrates optical frequency domain imaging (OFDI) — a high-resolution (~10 µm), volumetric diagnostic imaging tool now used clinically in Barrett's esophagus (BE) patients. This study aims to develop a versatile, OFDI-based thermal therapy monitoring platform to precisely target epithelial lesions, such as BE with dysplasia which is a precursor to esophageal adenocarcinoma (5-yr survival: ~15%). **Materials and methods:** We developed a microscopic thermal therapy guidance platform that combines imaging, radiofrequency ablation (RFA), and monitoring in a single OFDI-RFA balloon catheter configuration with direct clinical translational potential. A label-free, noninvasive technique to directly visualize the thermal coagulation process at high resolution was developed using complex difference variance (CDV), which exploits the dynamic microscopic fluctuations in the OFDI signals during thermal therapy. This is contrary to conventional, temperature-based RFA monitoring techniques, which are often invasive and only provide an indirect measure of tissue injury, or emerging techniques (e.g., MR and US thermometry) limited by their spatial resolution. **Results:** We demonstrated real-time, direct, label-free visualization of the coagulation process during RFA at high spatial and temporal resolution using our integrated OFDI-RFA balloon catheter system. Histological analysis using nitroblue tetrazolium chloride (NBTC) staining confirmed that the CDV-based technique accurately and directly delineates the thermal coagulation zone in porcine esophagus. **Conclusions:** While radiofrequency ablation is widely used for BE with high grade dysplasia, its use remains limited for earlier stages when the complete response rate is highly favorable (>90%). An important obstacle is that current RFA procedures can be highly variable (leading to recurrence and risk of complications such as stricture formation) as they incorporate minimal guidance for lesion identification using only *macroscopic* surface features assessed by conventional endoscopy and rely on pre-determined energy settings for dose delivery (assuming uniform lesion depth). The ability to directly and accurately visualize the thermal coagulation process at high resolution, as demonstrated in this study, enables the precise delivery of thermal energy to BE lesions (potentially enabling the use of RFA at earlier stages) and opens up the possibility of performing *microscopic* image-guided procedures in a vast array of epithelial applications beyond Barrett's esophagus in the new era of precision medicine.

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Effect of O-linked ß-N-acetyl-glucosamine Post Traumatic Brain Injury

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Traumatic brain injury (TBI) is the leading cause of death and disability worldwide. Of all types of injury, TBI is among the most likely to result in permanent disability and is a significant risk factor in the development neurodegenerative disease. There are currently no FDA-approved therapeutic agents for the treatment of TBI or TBI-induced neurodegeneration. It has been recently discovered that modification of proteins found in the cytosol and nucleus of cells by the O-linked attachment of ß-N-acetyl-glucosamine (O-GlcNAc) reduces cellular damage after injury. It has been shown that by increasing cellular O-GlcNAc, cell survival increases while reducing levels of O-GlcNAc is associated with increased cell death. Other research has shown that alterations in O-GlcNAc are key in the development of neurodegenerative disease. Currently, no other research has been conducted to test the effects of increasing O-GlcNAc after TBI. In this study, the effects of a new drug, thiamet-G**,** which increases O-GlcNAc in brain tissue, will be tested. The hypothesis of this experiment assesses if increasing O-GlcNAc levels in the brain with thiamet-G after TBI will reduce brain damage by protecting against synapse loss and deficits in synaptic function and reduce subsequent TBI-induced neurodegeneration.

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The Peripheral and Cortical Neural Basis of Tactile Texture Invariance

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Our sense of touch endows us with an exquisite sensitivity to surface texture. We can discern surfaces whose elements are tens of nanometers in size and hundreds of nanometers apart. The perception of texture not only allows us to make fine discriminations – like telling real silk from fake silk – but also guides object manipulation. Indeed, our perception of the surface properties of objects informs how much grip force we apply on them: more force is required for slippery objects. One of the remarkable aspects of tactile texture processing is that it operates over six orders of magnitude in element sizes, from the smallest discernible elements (on the order of tens of nanometers) to the largest elements that can fit on a fingertip, measured in tens of millimeters. We have shown that this wide range of scales is accommodated by distributing information across three types of nerve fibers, each sensitive to surface elements over different spatial scales. Importantly, these different nerve fibers convey texture information differently. Coarse textural features, on the order of millimeters, are conveyed in the *spatial* pattern of activation in one nerve fiber population, drawing analogies to visual texture representations on the retina. In contrast, fine textural features – with sizes in the tens of nanometers – are conveyed in *temporal spiking* patterns in two other nerve fiber populations, driven by skin vibrations elicited when the textured surface moves across the skin, and drawing analogies to audition. While nerve fiber responses are *highly dependent on exploratory parameters*, such as

contact force and scanning speed, the perception of texture is *highly invariant* with respect to these parameters. Thus, neural signals must be interpreted in the context of how they are acquired. Nothing is known about how this is achieved. To address this question, we recorded the responses of neurons in the primary somatosensory cortex (S1) of awake, behaving rhesus macaques as we scanned textured surfaces across their skin at different speeds. To test the degree to which neuronal responses are speed dependent, we used machine learning to classify textures based on the neuronal responses they evoked, pooling responses evoked by each texture at different speeds. To the extent that neuronal responses to a given texture were similar across speeds, classification performance would be high. Indeed, we found that the texture responses of neurons in S1 are independent of scanning speed as evidenced by high classification performance. In contrast, classification performance based on the responses of nerve fibers was poor unless speed-related differences in the responses were explicitly corrected for. We conclude that the perceptual invariance of texture across speed can be explained by neuronal responses at the earliest stages of cortical processing, namely S1.

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Transcriptional Networks of Resilience in Mouse Models of Depression: Upstream Regulators and Novel Avenues for Therapeutics

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Major Depressive Disorder (MDD) is a major contributor to morbidity and mortality worldwide. While research has identified a strong heritability of MDD, Genome Wide Association Studies (GWAS) have had limited success in identifying genetic alterations across populations. Consequently, researchers have turned to high-throughput RNA profiling techniques such as RNA sequencing to identify transcriptional changes associated with MDD. These efforts have been successful, identifying thousands of transcript alterations associated with MDD and depression-like behavior in animal models. However, research examining upstream regulators of these multiple transcriptional changes, which constitute strong candidates for novel antidepressant therapies, has been limited. **Methods:** We utilized the Chronic Social Defeat Stress (CSDS) model of depression to identify populations of mice susceptible and resilient to chronic stress. For each group, brain tissue was extracted and RNA from select depression-related regions was isolated for RNA sequencing. Sequencing data was used to generate both differential expression profiles and gene co-expression networks using Weighted Gene Co-Expression Network Analysis (WGCNA). Upstream regulators governing the resilience phenotype, which represents a natural homeostatic response, were identified and manipulated *in vivo* to determine their antidepressant effects. Further, resilient-specific transcriptional networks were cross-referenced with known drug transcriptional profiles to identify known therapeutics that could potentially be repurposed for MDD. **Results:** Upstream regulator analysis of our differential expression data identified Estrogen Receptor Alpha (ERα) as an upstream regulator of pro-resilient transcriptional changes. Viral overexpression of ERα in the nucleus accumbens (NAc) increased resiliency in both male and female stress models. In accordance with the predicted role of ERα as a transcriptional regulator, RNA sequencing of ERα-overexpressing NAc tissue mimicked the transcriptional profile of resilient mice. To complement this differential expression approach, we utilized WGCNA to identify a transcriptional network of co-expressed genes unique to the resilient phenotype. The most connected gene in this network, Zfp189, was also differentially expressed in CSDS. Viral overexpression of Zfp189 in the prefrontal cortex (PFC) was both pro-resilient and antidepressant. RNA sequencing of this PFC tissue identified transcriptional changes in the network from which Zfp189 originates, confirming the role of Zfp189 in regulating this co-expression network. Further, upstream regulator analysis suggested CREB, a transcription factor previously implicated in MDD, governs this network and its pro-resilient effects. To leverage this information for the development of novel MDD therapeutics, we cross-referenced our transcriptional networks with known drug transcriptional profiles and identified numerous candidates for novel antidepressants. Of these, three drugs appeared to induce antidepressant-like behavior in *in vivo* animal models. **Conclusions:** ERα is an upstream regulator of pro-resilient transcriptional changes and CREB-Zfp189 interactions regulate a resilient transcriptional network. Transcriptional findings such as these may be an effective starting point for the development of novel MDD therapeutics.

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Decoding the Lethal effects of *Staphylococcus aureus* Bi-component Leukocidins

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Staphylococcus aureus secretes many virulence factors that contribute to pathogenesis. One class of virulence factors is the bi-component pore-forming leukocidins. These toxins form pores in host target cells, often resulting in cell death. They have been shown to target neutrophils, red blood cells, and other hematopoietic cells in a receptor-dependent manner. Two members of the leukocidins, LukED and HlgAB, contribute to the lethality observed upon systemic infection of mice with *S. aureus*. Furthermore, either toxin is sufficient to cause rapid lethality in mice when injected intravenously. In this study we set out to understand the mechanisms by which these toxins exert this lethal effect, and the contribution of this effect to *S. aureus* bloodstream infection. We have observed that as mice succumb to intoxication their extremities become red, and their temperature drops precipitously. Moreover, we have found that vascular fluid accumulates in the skin of the mice, depleting fluid from the liver. Using intravital imaging, we have visualized the hemodynamic changes in the liver in real time. Histological examination shows that the vasculature of these mice becomes congested with red blood cells, suggestive of a hyper-acute form of shock. Experiments using mice deficient in critical pathways involved in inflammation and sepsis, together with mice deficient in several of the leukocidin receptors, suggest that the observed lethality is mediated through non-hematopoietic cells, and thus likely is the result of targeting of

previously uncharacterized target cell(s) or tissue(s). We are currently investigating the hypothesis that LukED and HlgAB are injuring the vasculature, and thus causing a massive leakage of vascular fluid and shock. Altogether, our study represents a potential new role for the bi-component leukocidins in *S. aureus* pathogenesis. *Conflict of Interest:* VJT, FA, and TRR are listed as inventors on patent applications filed by New York University School of Medicine, which are currently under commercial license to Janssen Biotech, Inc. The other authors declare that they have no conflict of interest.

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Early Exposure to Elevated IGF-1 Levels Increases Mammary Tumor Susceptibility through Expansion and Activation of the Mammary Stem Cell Compartment

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African American (AA) women suffer higher mortality of breast cancer (BrCa) than White women of European descent. Assessment of this disparity reveals that AA women are more likely to develop the early onset, treatment refractory, triple negative (basal) BrCa subtype associated with worse prognosis. The mechanistic basis for the difference in development of BrCa subtypes remains unresolved, but it has been shown that young AA girls have significantly higher circulating levels of Insulin-like growth factor 1 (IGF-1) than their age-matched White counterparts, implicating early exposures to this mitogenic and pro-tumorigenic growth factor in mediating BrCa subtype. To investigate the role of IGF-1 in mammary tumorigenesis, we use the transgenic (Tg) BK5.IGF-1 model, which recapitulates the paracrine effects of IGF-1 exposure on the mammary epithelium. We previously showed that exposure to elevated levels of IGF-1 are strongly pro-tumorigenic in the mammary gland, and pre-pubertal Tg mice have an increased number of terminal end buds, which are known to be important stem cell niches. In this study, we found that the mammary stem cell (MaSC) pool was expanded in both pre- and post-pubertal Tg mice compared to age-matched WT animals. Flow cytometry and immunolocalization identified the expression of IGF-1R on both WT and Tg MaSCs. Single-cell transcriptomic analysis of MaSC compartment revealed that IGF-1 stimulated Cyclin D1 (*Ccnd1*) gene expression and increased the proliferation of "activated" transit stem cells (Tr-MaSCs). Moreover, Gene Set Enrichment Analysis (GSEA) demonstrated that genes involved in stemness, proliferation, EMT, invasion and metastasis were highly upregulated in Tr-MaSCs from Tg mice compared to age-matched WT animals. This may also reflect the program by which quiescent-stem cells undergo commitment and exit from the niche. Previous studies suggest that loss of cell polarity is associated with increased stem cell self-renewal and susceptibility to tumorigenesis. Interestingly, we also demonstrated downregulation of genes associated with cell polarity in Tr-MaSCs from Tg animals, suggesting an increased number of stem cells undergoing symmetric cell divisions. Overall, our results identify a novel tumorigenic mechanism, by which early exposure to IGF-1 expands the MaSC compartment and “primes” these cells for transformation, thereby increasing mammary tumor incidence and reducing latency.

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Glucocorticoids Depress Intestinal Stem Cell (ISC) Activation and Proliferation Required for Mucosal Restitution in Inflammatory Bowel Disease

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In the treatment of inflammatory bowel disease (IBD), achievement of mucosal healing remains a reliable predictor of durable remission. For moderate to severe IBD, exogenous glucocorticoid steroids (GC) are the preferred treatment due to their cost and efficacy. Despite the clinical benefits, endoscopic data show that GCs delay ulcer healing in more than 30-percent of patients through poorly understood mechanisms. Intestinal stem cells (ISC) require Wnt/β-catenin signaling for maintenance of the crypt proliferative compartment, and our data suggest Wnt-responsiveness is an important component for mucosal healing. Therefore, we posit that promoting ISC activation in response to epithelial injury should be a goal for therapy. The objective of our study was to determine whether GCs regulate ISC activation in ulcer re-epithelialization. Specifically, we hypothesized that that steroids impair Wnt/β-catenin signaling required for ISC activation during mucosal repair. Methods: We examined the effects of dexamethasone, a GC steroid, on cell signaling pathways involved in Wnt/β-catenin, PI3 Kinase, and NFkB signaling. We analyzed changes in gene expression and subcellular protein in NCM460 (NCM) cells, a normal human colonic intestinal epithelial cell (IEC) line, mouse colonic organoids, IEC from DSS colitis mice and human epithelial isolations from patient biopsies. Results: In steroid-treated NCMs, we saw a significant decrease in transcript levels of Wnt target gene Axin2, a marker of Wnt-responsive multipotent IECs that participate directly in ulcer healing. Further, these data paralleled results from steroid-treated mouse organoids, which showed significant reductions in mRNA for stem cell markers Lgr5 and Ascl2, proliferative markers Ki67 and PCNA, and Wnt-targets Axin2 and CTNNB1. After transfecting NCM460s with lentivirus containing a TCF/LEF luciferase construct (a marker of Wnt transcriptional activation), we saw that steroid-treatment diminished the 2.5-fold TNF-mediated increase in TCF/LEF signaling back to control levels. In both murine and patient-derived IECs, GCs inhibited the colitis-induced increases in Axin2, p-LRP6 (marker of Wnt signaling) and nuclear localization of β-catenin protein levels. Together, these data indicate strongly that GCs impair Wnt signaling in stem cells. Discussion: Our results indicate that GCs impair Wnt/β-catenin signaling during ulcer healing in in vitro and in vivo models of colitis. While steroids play a significant role in regulating inflammatory immune responses in IBD, our data suggest steroids inhibit essential components of epithelial signaling needed for ulcer healing. These data also suggest that novel topical therapies that promote Wnt signaling may accentuate ulcer healing in colitis patients resistant to steroids.

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Elucidating the Genetic Determinants for Exit out of Pluripotency with a CRISPR-Cas9 Genome-wide Knockout Screen

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Early mammalian embryogenesis is characterized by robust cellular proliferation, maintenance of pluripotency, and resistance to differentiation until gastrulation, when cells must commit to specific lineages. Pluripotent stem cells in the early mammalian embryo progress through developmental states in preparation for lineage specification during gastrulation, but how pluripotent stem cells become competent to differentiate is not well understood. 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. The naïve state and primed state cells recapitulate pluripotent cells that are resistant to differentiation and competent to differentiation, respectively. Naïve ES cells can rapidly transition to the EpiLC, primed state with simple changes to cell culture 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 40 high confidence candidates (FDR < 10%), 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. This proposal aims to confirm my screen findings and elucidate roles for late-endosome-lysosome fusion and mTOR pathway/autophagy in exit from the naïve state. Demonstrating these roles will provide significant new insight into how cytoplasmic processes function to enable pluripotent cells to become competent to differentiate. Therefore, the findings of this proposal will be critical to understanding both basic developmental biology as well as deriving application from pluripotent stem cells for regenerative medicine and cell-based therapies. > < 10%), 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. In confirming the screen findings, this work has elucidated roles for late-endosome-lysosome fusion and mTOR pathway in exit from the naïve state. Demonstrating these roles has provided significant new insight into how cytoplasmic processes function to enable pluripotent cells to become competent to differentiate. These findings are critical to understanding both basic developmental biology as well as deriving application from pluripotent stem cells for regenerative medicine and cell-based therapies.

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Mapping Antibiotic Targets in Biofilms to Develop Better Therapies for Cystic Fibrosis

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The most important clinical obstacle in cystic fibrosis (CF) is treatment failure due to biofilms. Biofilms are a community of sessile cells enmeshed in a thick gel matrix that leads to thousands of times more resistance to antibacterial therapies, macrophages, and neutrophils. A hallmark of CF is a defective mucociliary transport system that results in dry mucus production and clogged airways, creating an environment that is ideal for colonization by *Pseudomonas aeruginosa*. Central to this pathogen's success is its biofilm mode of growth within the lungs of CF patients, which are essentially impossible to eradicate with current antibacterial therapies, leading to immune complex-mediated chronic inflammation, neutrophilic tissue damage, decreased lung function, and ultimately death. Using a high throughput screen on 6,090 compounds from a drug repurposing library, we identified 60 hits that enhance the killing of *P. aeruginosa* biofilms by the first-line drug tobramycin. One combination, tobramycin and triclosan, eradicated greater than 98% of the strain PAO1 *in vitro* even though neither treatment was effective on its own. To understand this activity, it is critical to ascertain the mechanism of action of this treatment. Targeted experiments ruled out a number of potential mechanisms based on the known activities of triclosan and tobramycin. Therefore, I utilized *"evolution in action"* to evolve mutants that are resistant to tobramycin/triclosan. Biofilms were grown on MBEC™ pegs for 24 hours and then treated with tobramycin/triclosan for 24 hours. To recover viable cells from the biofilm, the MBEC pegs were sonicated for fifteen minutes and cells surviving the treatment were recovered in fresh media overnight. The recovered resistant cells were re-grown as biofilms on MBEC pegs and another cycle of treatment was performed. In each subsequent cycle, an ever-increasing concentration of tobramycin and triclosan was used. By gradually increasing the selection pressure, I evolved 191 single colony isolates that are ~200x more resistant triclosan/tobramycin. Next, to determine the molecular mechanism of resistance, Illumina HiSeq was performed. Using Breseq, we expect to identify mutant genes responsible for resistance to tobramycin/triclosan, yielding insights into the mechanism of action of this combined therapy. Evolution is a powerful tool that can elucidate the mechanism of action of new therapies and yield insights into how resistance develops inbiofilms—potentially mapping new antibacterial targets for the treatment of CF.

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TRAF3 is a Negative Regulator of a Pim2 in B Cells

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TRAF3 is a versatile intracellular protein that has multiple cell-type and context-specific roles. In B cells, loss of TRAF3 results in an aberrant survival phenotype that increases risk of cell transformation and lymphomagenesis. The mechanism by which TRAF3 regulates B cell death is not well defined. In this study, we show that loss of TRAF3 resulted in the induction of the pro-survival kinase Pim2 in B cells independently of non –canonical NFκB. TRAF3-deficient B cells and multiple myeloma cells displayed higher susceptibility to Pim inhibition. In contrast, TRAF3 deficiency rendered cells resistant to inhibitors of the PI3K/Akt pathway. Loss of TRAF3 also led to transcription-independent c-Myc elevation that was dependent on increased Pim2 and decrease in c-Myc ubiquitination. Overexpression of c-Myc in mouse B cells resulted in Pim2 induction, suggestive of a positive feedback loop in the absence of TRAF3. TRAF3 deficiency made B cells resistant to the c-Myc inhibitor JQ1, but the drug enhanced Pim inhibitor-mediated killing. Our results show that TRAF3 suppresses a Pim2/c-Myc positive feedback loop that promotes B cell survival.

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The Novel and Safe Pharmaceutical Compound OLT1177 Reduces Inflammation by Preventing Activation of the NLRP3 Inflammasome

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Formation of the NLRP3 inflammasome following infection or tissue injury induces maturation of IL-1β, a validated target for a broad spectrum of acute and chronic inflammatory diseases. We identify the mechanism of action of OLT1177, an orally active and human tested β-sulfonyl nitrile synthetic molecule. In healthy humans, OLT1177 is safe at oral doses up to 1000 mg each day for 8 days, with no clinical, hematological or organ toxicities observed. OLT1177 is currently being evaluated as oral treatment for acute gout flares in a Phase 2 clinical study. In human blood-derived macrophages OLT1177 reduced processing and release of IL-1β (-60%; $P < 0.001$) and IL-18 (-70%; $P < 0.001$) by preventing NLRP3 inflammasome formation measured by Fluorescent Resonance Energy Transfer (FRET; $P < 0.01$). In freshly obtained human blood neutrophils, OLT1177 inhibited processing and release of IL-1β by 58% at 1μM and reduced caspase-1 activity ($P < 0.01$) following LPS and ATP stimulation. In murine macrophages cell line, J774A.1 cell, OLT1177 showed to reduce IL-1β release at nanomolar concentrations ($P < 0.001$), caspase-1 activity (-35%; $P < 0.01$) and caspase-1–dependent cell death pyroptosis ($P < 0.05$) following inflammasome activation. The release of TNFα was no affected by OLT1177. The compound had no effect on the transcription of *il1β*, *il18*, *caspase1*, *asc* or *nlrp3* suggesting to prevent IL-1β maturation and not the priming phase. Measurement of membrane ion current following P2X7 receptor activation with ATP using patch clamp and two-electrode voltage-clamp (TEVC) analysis showed that OLT1177 have no effect on K^+ efflux, thus to target downstream of the P2X7 receptor. Parallel to the inhibition of canonical and non-canonical NLRP3 inflammasome, OLT1177 showed no effect on AIM2 and NLRC4 inflammasomes. In healthy humans, blood levels of OLT1177 following oral administration exceeded by 100-fold the concentration that reduces processing and secretion of IL-1β and IL-18 from human cells *in vitro*. *In vivo*, OLT1177 limited the severity of LPS-induced systemic inflammation with reduction in peritoneal fluid levels of IL-6 by 44%, MPO by 80% and CXCL1 by 30% ($P < 0.05$) in mice. Metabolomics analysis of muscle from mice treated with parenteral administration of endotoxin showed that OLT1177 reduced oxidative stress and increased total levels of adenylate metabolites (ATP, ADP and AMP). Consistent with a reduction in oxidative stress, levels of TCA cycle intermediate αketoglutarate were increased in OLT1177-treated animals ($P < 0.05$). Treatment with OLT1177 also showed accumulation of citrate and decreased levels of oxaloacetate ($P < 0.05$). These data are indicative of activation of early oxidative reactions of mitochondrial metabolism. Parallel with increased oxidative metabolism, tissue levels of pro-inflammatory marker succinate were decreased in response to OLT1177 treatment ($P < 0.05$). In conclusion OLT1177 is a novel, safe and specific inhibitor of the NLRP3 inflammasome, with the potential to be a lead compound for the treatment of IL-1β and IL-18-mediated diseases.

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Macrophage Heterogeneity as a Driver of The Age-Related Susceptibility to Influenza A Infection

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Seasonal influenza A virus infection causes at least 20,000–50,000 deaths in the United States each year and causes disproportionate morbidity and mortality in older individuals. We and others have found similarly disproportionate mortality in aged mice infected with influenza A. An important role for alveolar macrophages in the response to influenza A infection is recognized. Strategies to therapeutically target alveolar macrophages during lung injury have considered that they are a single population of monocyte-derived cells. This paradigm has been challenged by studies that identified tissue-resident alveolar macrophages (TR-AM) as highly specialized cells that populate the lung shortly after birth and persist over the lifespan. **Methods**: We aged bone marrow chimeric C57Bl/6 (CD45.1 donor into CD45.2 host) mice generated with thoracic shielding followed by busulfan depletion of the remaining bone marrow. The resulting chimeric mice (>99% donor monocytes in blood, >99% recipient in tissue resident alveolar macrophages), were treated with influenza A (A/WSN/33) virus, or bleomycin. **Results:** Severe injury early in life alters the alveolar macrophage landscape during aging. We treated shielded-bone-marrow chimeric mice with influenza A or bleomycin and measured the ratio between Mo-AM and TR-AM 10 months later. We found that TR-AM were remarkably stable, constituting >95% of alveolar macrophages in naïve mice at 14 months of age. In contrast, in mice infected with influenza A (A/WSN/33) virus, 100pfu at 8 weeks post bone marrow transfer (4 months of age), approximately 50% of the alveolar macrophages were monocyte-derived 10 months later. To determine whether this finding was unique to influenza A, we treated a separate cohort of shielded-bone-marrow chimeric mice with bleomycin and obtained similar results. Next, we sought to determine whether the differences in gene expression between TR-AM and Mo-AM we observed during injury and fibrosis persisted over the lifespan. Ten months after

bleomycin treatment or influenza A infection, TR-AM and Mo-AM were not distinguishable by flow cytometry. A comparison of the transcriptomes of TR-AM and Mo-AM 10 months after bleomycin administration revealed only 101 differentially expressed genes. **Conclusion:** This finding suggests that a severe injury early in life can permanently reshape the alveolar macrophage landscape with respect to its developmental origins. If monocyte-derived alveolar macrophages differ in their response to challenge, this might explain some of the age-related susceptibility to influenza A infection.

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Induced MHCII Expression on Breast Cancer Cells Broadens the Responding T cell Repertoire, Delays Tumor-specific T Cell Exhaustion, and Impairs Tumor Growth

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We recently reported that the aberrant expression of Major Histocompatibility Class II (MHCII) molecules on human triple negative breast cancer (TNBC) cells correlates with prolonged progression-free survival and increased tumor infiltrating lymphocytes. We hypothesized that the expression of MHCII enhances the intratumoral CD4+ T cell response, thereby bolstering the tumor-specific CD8+ T cell response, resulting in more effective tumor control. To test our hypothesis, we created both MHCII-expressing and MHCII-negative tumor cells by transfecting murine breast cancer (TS/A) cells with the human class II transcriptional activator (hCIITA) or empty vector, respectively. Transfected cells were then injected into BALB/c mice and the resulting immune response analyzed by flow cytometry at four time points. We found that hCIITA-expressing tumors grew slower than control tumors in immunocompetent recipients, but that this difference was nullified in immunocompromised and markedly reduced in CD4+ T cell depleted mice. CD4+ T cells isolated from hCIITA-transfected tumors produced more IFNγ for longer times than their counterparts in control tumors. Similarly, CD8+ T cells isolated from hCIITA-transfected tumors displayed a more activated phenotype and produced more IFNγ and granzyme B for longer times. Nevertheless, both CD4+ and CD8+ T cells eventually became exhausted in both groups. In addition to enhanced effector functions, TCR repertoire analysis demonstrated that both the breadth and magnitude of expansion of responding T cell clones were increased in hCIITA-transfected tumors. Interestingly, transfected and non-transfected tumors selected for regulatory T cells of distinctly different phenotypes. Finally, we show that the histone deacetylase inhibitor Entinostat is capable of robust and dose-dependent induction of MHCII on tumor cells in vivo, an effect that correlates with dramatic reduction in tumor size. These results suggest that the clinical benefit associated with MHCII expression on TNBC cells is mediated by a delay in T cell exhaustion and increased intratumoral CD4+ T cell activation, which enhances the cytotoxic capacity of CD8+ T cells. Entinostat, and potentially other epigenetic modifying agents, may enable induction of MHCII expression on TNBC cells clinically and allow more patients to benefit from an augmented T cell response. These effects may be magnified by combinatorial therapy with checkpoint inhibitors to promote durable anti-tumor immune responses.

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The Role of the Neurodegeneration-associated RNA/DNA Binding Protein FUS in Mitochondrial Function

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Fused in Sarcoma (FUS) is a multifunctional DNA/RNA binding protein known to be involved in diverse processes of RNA and DNA regulation, including splicing, RNA transport, RNA stability, and DNA repair. Since 2009, it's been known that intracellular inclusion bodies containing FUS are the pathological hallmark of a subset of cases of Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Lobar Degeneration (FTLD). These cases have since been reclassified as FUS proteinopathy. Despite much recent effort to study FUS proteinopathy, the biological function of FUS in the nervous system remains unknown, and molecular mechanisms underlying the pathogenesis of FUS proteinopathy remain to be elucidated. Our group recently demonstrated that overexpression of wild-type or ALS-mutant FUS results in mitochondrial morphological changes and functional impairment, with similar morphological defects observed in the brains of FTLD-FUS patients. Our results raised the question of whether endogenous FUS plays a role in mitochondrial biogenesis and function. Here we examined the effect of knocking out or knocking down FUS on mitochondrial morphology and function, using a combination of bioinformatics, molecular biology, biochemistry and imaging. Our experiments suggest that FUS plays a role in mitochondrial biogenesis. We generated an mRNA-Seq dataset from whole brains taken from FUS KO mice and their wild-type littermate controls, and identified several candidate FUS-regulated mitochondrial-associated genes. We then re-analyzed several previous RNA-Seq studies of FUS KO or FUS knockdown in neurons and identified a number of mitochondrial-associated genes and pathways that were shared across most of the datasets. These candidate FUS-regulated gene pathways had not been systematically analyzed in the original publications. We have begun to validate the expression of a subset of these putative FUS-regulated mitochondrial genes in both the HEK FUS KO and the primary rat cortical neuron FUS knockdown model systems. At the time of submission, we were working on manipulating the expression of these genes to demonstrate a rescue of the mitochondrial defects observed. Our work indicates that FUS regulates mitochondrial morphology and function, and that mitochondrial defects are an early event in FUS proteinopathy. Our study also suggests that both gain-of-toxicity and loss-of-function mechanisms may contribute to the pathogenesis of these fatal diseases.

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Quantitative Susceptibility Mapping using Echo Planar Magnetic Resonance Imaging

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New medical imaging technologies have the potential to change how doctors diagnose patients. As these technologies improve, diagnoses can be made earlier, leading to improvements in health outcomes. Quantitative Susceptibility Mapping (QSM) is one of these technologies. QSM uses post-processing to measure susceptibility sources within an MRI image. Typically, the phase shifts from the MRI data are first separated into background and local phase shifts. Then the local phase shifts are converted into local susceptibility sources, producing a medical image, or map, of the susceptibility sources. Applying QSM to Echo Planar MRI images led to artifacts in the images. Herein, we analyze the images to determine what the source of these artifacts are, and use processing techniques to remove said artifacts.

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Discrete Populations of Mononuclear Phagocytes Orchestrate the Initiation and Progression of Mitral Valve Disease by Mediating Systemic and Local Inflammatory Processes

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The mitral valve is the most frequently diseased heart valve. Nearly every case of MV disease displays abnormal remodeling and evidence of chronic inflammation such as a predominance of myofibroblasts and fibrosis. Thus, mitral valve disease (MVD), like most forms of acquired cardiovascular disease (CVD), is chronic, systemic, inflammatory, and fibrotic. A comprehensive understanding of the mechanisms mediating cardiovascular remodeling during chronic inflammation does not exist, however. Herein, we build on previous work to demonstrate discrete populations of mononuclear phagocytes orchestrate MVD through remote induction of systemic inflammation and through local programming of MV infiltration. **Methods.** KRN T cell receptor (TCR) transgenic mice were crossed with C57BL/6 mice congenically expressing the I-Ag7 major histocompatibility complex II (MHC-II). The progeny of this cross develop fully penetrant fibro-inflammatory MVD beginning at 3 weeks of age. All studies were conducted using this genetic background. Monoclonal antibody (mAb) neutralization was used to probe hypothetical disease-contributing elements. At disease onset, mAb (or species-matched isotype controls), were injected into the peritoneal cavity twice weekly for four weeks to neutralize the following proteins: tumor necrosis factor, TNF; interleukin-6, IL6; vascular cell adhesion molecule-1, VCAM1; very late antigen-4, VLA4. Histological assessment of valve remodeling was conducted thereafter. Conditional deletion in the monocyte lineage was achieved by crossing mice expressing Cre under control of the CX3CR1 promoter to lines with target alleles floxed. To render the monocyte lineage insensitive to activating Fc gamma receptor signaling, mice floxed at the Syk locus were employed. To prevent monocyte recruitment from the vasculature to inflamed tissues, mice floxed at the CD49d (Itga4/α4 integrin) locus were used. In all cases, Cre-negative littermate controls were employed. Standard histological staining and flow cytometry methods were used in all cases. **Results.** Due to the persistence of systemic inflammation in K/B.g7 mice, the chronically inflamed valves become fibrotic and abnormally remodeled. With a combination of in vivo experimental approaches, we demonstrate mitral fibrosis is initiated by inflammatory cytokines (TNF, IL6) produced by mononuclear phagocytes downstream of an FcγR-Syk signaling axis within secondary lymphoid tissue. This systemic inflammatory process results in localized activation of the mitral stroma leading to upregulation of VCAM1 at the blood-valve interface. Circulating inflammatory monocytes expressing CD49d/CD29 (VLA4, α4β1integrin) are subsequently recruited to the inflamed tissue site. **Conclusions**. These data provide a novel perspective on the role for Fc receptors in the development of atherosclerosis. Additionally, they demonstrate the presence of a tissue-specific pathology mediated by a systemic inflammatory process. Finally, these studies identify future potential therapeutic targets (e.g. Syk) that could be explored for the treatment of CVD. Ongoing studies intend to identify how macrophages recruited to inflamed mitral valves contribute to downstream matrix production and fibrosis.

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Genetic And Molecular Differences Between Combined and Isolated Post-Capillary Pulmonary Hypertension

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Pulmonary hypertension is most commonly caused by elevated left ventricular (LV) filling pressure due to LV systolic, diastolic, or valvular dysfunction (PH-LHD). PH-LHD may be complicated by superimposed pulmonary vasculopathy, resulting in combined pre- and post-capillary pulmonary hypertension (Cpc-PH). Although associated with increased morbidity, Cpc-PH has no specific therapy. Growing evidence suggests that Cpc-PH has a pathophysiology distinct from isolated post-capillary PH (Ipc-PH). We have previously demonstrated increased endothelin 1 (ET-1) in blood sampled from the pulmonary artery wedge position of patients with Cpc-PH compared with Ipc-PH, and correlation between wedge ET-1 and pulmonary vascular resistance only in the Cpc-PH group. In this study, we leveraged an electronic medical record-linked DNA biorepository to test the hypothesis that genetic variation in the ET-1 system and other pathways relevant to pulmonary vascular biology contribute to Cpc-PH pathology. **Methods**: We identified patients with Cpc-PH and Ipc-PH by extracting hemodynamic and clinical data from patients referred for right heart catheterization from 1998-2014. Patients with existing genotyping on the Illumina Human Exome BeadChip were analyzed (Cpc-PH n=36, Ipc-PH n=139). First, we targeted reported genes involved in ET-1 synthesis and signaling. Next, we performed an unbiased analysis, identifying single nucleotide variants (SNVs) with minor allele frequency >5% differentially represented between Cpc-PH and Ipc-PH with nominal statistical significance ($p < 0.05$). These genes were used to perform pathway analysis using WebGestalt and their relative tissue expression was evaluated using GTEx. **Results**: SNVs resulting in missense or nonsense mutations were present in 37 genes involved in ET-1 synthesis and function. Several of these SNVs approached statistical significance when comparing Cpc-PH and Ipc-PH in this limited sample, including ECE1 (endothelin converting enzyme 1, odds ratio 2.2, p-value 0.08) and EDNRB (endothelin receptor type B, odds ratio 3.9, p-value 0.19). In the exploratory

unbiased analysis, 770 SNVs in 669 genes were differentially associated with Cpc-PH vs. Ipc-PH, including pathways previously associated with pulmonary vascular pathology such as cytoskeletal and extracellular matrix function. In the genes with tissue expression reported in the GTEx database, there was 34% higher expression in the lungs (3rd highest expression of 52 tissues) relative to mean tissue expression, supporting biologic plausibility for their role in Cpc-PH. **Conclusion**: In this exploratory study comparing genetic differences between Cpc-PH and Ipc-PH, we identified pathways overrepresented in Cpc-PH and known to be perturbed in pulmonary vascular disease. These findings provide further evidence for a distinct pathophysiologic etiology and perhaps a genetic predisposition for Cpc-PH. SNVs in the ET-1 system associated weakly with Cpc-PH, highlighting the need for larger sample sizes. Further work is warranted to replicate and evaluate the functional consequences of candidate genetic variants identified in this study.

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Development of Biligand Capture Agents to Target *Plasmodium falciparum* Histidine-Rich Protein II (*Pf*HRP2) for Rapid Malarial Diagnostics

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Currently, malarial rapid diagnostic tests use antibodies to detect *Plasmodium falciparum* histidine rich protein II (PfHRP2), a biomarker for *Plasmodium falciparum* malarial infection. Malaria caused by this species is the most dangerous form of malaria and has the highest rate of fatality. However, the antibodies required for these tests are too costly and too chemically unstable to be used viably in third world countries. Thus, we are working to develop the technology for an antibody-free rapid malarial diagnostic test which will use peptide capture agents to target the entire PfHRP2 protein. Macrocyclic peptides against distinct epitopes of PfHRP2 have been developed. We are aiming to develop capture agents with higher binding affinity than the monoligands in order to develop an accurate test with a low limit of detection. We want to increase the binding affinity of the capture agents by linking two PfHRP2 monoligands to create a biligand. In doing so, we want to test a cooperativity hypothesis to see if binding multiple sites on the PfHRP2 protein simultaneously with an optimized linker will yield better affinity. The PfHRP2 binding affinity of the biligands was evaluated and compared to the monoligands through colorimetric enzyme-linked Immunosorbent assays (ELISAs). My presentation describes in detail the procedure used to synthesize these biligands, along with the process to find the best biligand binders for PfHRP2.

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Directed *in vitro* Evolution of MyoD for the Development of Engineered Myoblasts for Enhanced Cell Transplantation

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Current treatment options for skeletal muscle diseases are largely limited to inflammatory symptom management and physical therapy. Although promising as an alternative treatment strategy, cell therapy for skeletal muscle regeneration has been marred by host immune rejection, limited migratory ability, and acute cell death of transplanted myoblasts. Our central hypothesis is that MyoD can be engineered to directly reprogram myoblasts with superior skeletal muscle functional characteristics and engraftment potential. The rationale for the proposed research is that generating autologous skeletal myoblasts with enhanced myogenic potential and fusion capacity will promote successful engraftment and potentially reopen cell transplantation as a viable avenue for skeletal regenerative therapy. Previous studies have demonstrated that rational engineering of MyoD enhances the conversion of human dermal fibroblasts and adult mesenchymal stem cells into myoblasts. We hypothesize that unbiased engineering using an established directed evolution approach would generate a "super" MyoD capable of robust myogenic conversion, and provide valuable insights into the central elements of lineage conversion. Error-prone PCR will be used to create a library of MyoD mutants that will be screened for enhanced transdifferentiation by FACS, and optimal mutants will feed into subsequent cycles of evolution. We suspect that our generated mutants can greatly enhance the fusion capacity of reprogrammed myoblasts, resulting in improved *in vivo* engraftment potential. Engineered myoblasts will be assessed for fusion competence by immunocytochemistry, fusion assays, and engraftment *in vivo*. This work will unveil critical insights into the transcriptional and molecular determinants of skeletal myoblast reprogramming and engraftment. In turn, these insights may reinvigorate the field of myoblast transplantation as a viable avenue in skeletal muscle regenerative therapy.

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Tumor Derived Exosomes Induce Formation of a Pre-Metastatic Niche in Lung Cancer via Polarization of Macrophages and γδ T cells towards an Immunosuppressive PDL-1 Expressing Phenotype

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Exosomes and PDL-1 expression, two hot topics in the field of Immunotherapy, but can they be related? Exosomes are small endocytic vesicles ranging in size from 30-120nm that are secreted from both normal and pathological tissues. Programmed Death Ligand-1, (PDL-1) is an immune checkpoint inhibitor which inactivates tumor infiltrating CD8+ T cells. In the context of tumor metastasis, it has been shown that exosomes are capable of influencing formation of a metastatic niche through delivery of immunosuppressive factors like TGF-β and oncogenic microRNAs. Similarly, intravenously injected breast cancer derived exosomes were internalized by macrophages in the lung and brain further demonstrating their ability to modulate the tumor microenvironment. The question though still remains as to what are the effector molecules being regulated by the exosomes and do the exosomes play a role in PDL-1 induced T-cell anergy. **Objective**: Using the LLC lung cancer model, we hypothesize that tumor derived exosomes are originally engulfed by alveolar macrophages, increasing their expression of PDL-1 and polarizing them towards an M2 phenotype. Additionally, we hypothesize that the exosomes also have a direct effect on the lung γδ T cell population causing them to differentiate into immunosuppressive γδT17 cells. It recently has been shown that tumor infiltrating γδT cells are capable of restricting αβ T cell activation in a pancreatic ductal carcinoma model. Furthermore, IL-17 within the tumor microenvironment is linked to recruitment of MDSCs, tumor infiltrating neutrophils, and angiogenesis. Therefore, the combination of the immunosuppressive M2 macrophages and the pro-tumor functions of γδT17 results in the formation of a metastatic niche. **Methods**: Exosomes are isolated via the classical ultracentrifugation method. They are then co-cultured with whole lung cell homogenate to determine effect on the myeloid cell populations, γβ T cells and αβ T cells. M2 polarization is determined by surface marker expression and CFSE OT-I/II proliferation assay. Using siRNA, microvesicle production was inhibited in a GFP LLC cell line that was i.v. injected into BL/6 mice to determine effect on metastatic kinetics and lung cell populations. **Results**: In various tumor models, LLC, LKR, p53/KRAS, exosomal stimulation was shown to increase PDL-1 expression and promote M2 polarization. Additionally, exosomally stimulated γδ T cells regulate αβ T cells through the PD-1/PDL-1 axis and induce effector function of γδT17 cells, namely IL-17 secretion and MDSC recruitment. **Conclusion**: Exosomes increase PDL-1 expression on M2 macrophages and polarize γδT17 cells to an immunosuppressive phenotype that restricts αβ T cells resulting in the formation of a pre-metastatic niche.

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Endothelial Mineralocorticoid Receptors Contribute to Sex-Dependent Vascular Inflammation in Atherosclerosis

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Complications of atherosclerosis are the leading cause of death in the USA. Ischemic events, including heart attacks and strokes, are predominantly caused by rupture and thrombosis of inflamed, "vulnerable" atherosclerotic plaques. Clinical studies show that excess of the hormone aldosterone predisposes to cardiovascular ischemia and that inhibition of the aldosterone-binding mineralocorticoid receptor (MR) prevents adverse outcomes. We previously showed that vascular endothelial cells (ECs) express MR, which contributes to leukocyte adhesion to ECs in culture. We hypothesize that EC-specific MR promotes vascular inflammation in hyperlipidemia, thereby contributing to the vulnerability of atherosclerotic plaques. **Methods and results:** We first investigated the effect of whole-body MR inhibition with spironolactone (Spiro) on vascular inflammation in male ApoE-KO athero-prone mice fed high fat diet (HFD) for 8 weeks. Preliminary flow cytometry results quantifying specific leukocyte populations in the aortic arches reveal that Spiro reduces total leukocytes, T cells, and monocytes within the aortic arch by up to 80% compared to placebo. *In vitro* treatment of human coronary artery ECs with oxidized phospholipids to mimic atherogenic conditions increased leukocyte adhesion by 50% ($p < 0.01$), which was blocked by Spiro pretreatment. This suggests that EC-MR contributes to leukocyte adhesion in dyslipidemia. To explore the role of EC-MR in vascular inflammation *in vivo*, transgenic mice with EC-specific MR deletion (VE-Cadherin-Cre/MR-flox) were injected with an adeno-associated virus expressing human PCSK9 and fed HFD for 12 weeks, resulting in hyperlipidemia (cholesterol>1000 mg/dL) and atherosclerosis. There was no difference in serum cholesterol, fasting glucose, body weight gain, blood pressure, or serum aldosterone levels between EC-MR-KO and MR-intact mice of the same sex. Histologic analysis of plaques in the aortic root revealed that EC-MR-KO did not affect overall plaque size. In EC-MR-KO mice of both sexes, plaque lipid content tended to be lower ($p=0.09$), a phenotype consistent with more stable plaques with EC-MR-KO. Quantitative flow cytometry analysis of aortic arches revealed that EC-MR-KO males had a 40% reduction in total leukocytes ($p < 0.05$) and monocytes ($p=0.05$) compared to controls, with a trend towards fewer T cells ($p=0.09$). MR-intact females had 50-60% fewer intravascular leukocytes, T cells, and monocytes than MR-intact males ($p < 0.01$), and this was not further decreased by EC-MR KO. **Conclusions:** MR inhibition attenuates vascular inflammation in male mice. Endothelial MR contributes directly to leukocyte adhesion to ECs *in vitro* and to inflammation *in vivo* in dyslipidemic males. MR-intact females are protected from vascular inflammation. Our results suggest a new mechanism for the higher incidence of cardiovascular ischemia and death in MR-activated patients and for sex differences in the incidence of cardiovascular ischemia. Further studies are underway to determine the mechanisms for the proinflammatory role of EC-MR in atherosclerosis and for the observed sex difference.

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Chimeric Antigen Receptor T-Cell Therapy of Differentiated Thyroid Cancer

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Thyroid cancer presents a pertinent clinical challenge as the most common endocrine cancer, accounting for 3.6% of all new onset cancers and the third most common solid tumor malignancy in children. Papillary (80%) and follicular (15%) thyroid cancer arise from differentiated tissues, and some cases of differentiated thyroid cancer (DTC) are refractory to current treatment modalities including thyroidectomy and 131-iodine radiotherapy. In pediatric populations, the radioactive dose of 131-iodine may cause future complications, discouraging its use. As an additional approach to thyroid cancer treatment, we investigate use of the fourth-generation Chimeric Antigen Receptor T cell (CAR-T) construct. Use of immunotherapy for cancer treatment is supported by recent clinical trials in ALL, osteosarcoma, and melanoma, suggesting that CAR-T may be an effective approach against solid tumors. Directing the CAR-T system against extracellular targets on thyroid cancer cells spurs further progress and innovation in pediatric and adult thyroid cancer treatment. We hypothesize that CAR-T cells targeted against extracellular receptors on papillary and follicular thyroid cells will specifically target and ablate thyroid tissue including well-differentiated thyroid cancer cells. **Methods:** We established three CAR-T cell lines with anti-TSHR (thyroid stimulating hormone receptor), anti-Tg (thyroglobulin) and anti-NIS (Na^+/I^- symporter) . By co-culturing GFP-positive tumor cell lines with CAR-T cells and negative controls, cell killing assays were performed to determine effect of extracellular motif-targeting CAR-T induced apoptosis. T cells are prepared from full blood samples, then cultured with lentivirus to express the anti-target CAR ectodomain composed of a single chain variable fragment derived from a monoclonal antibody as well as CD28 and ζ-endodomains to mimic costimulatory T cell activation. GFP-positive thyroid cultures prepared for cell killing assays include the papillary thyroid cancer K-1 cell line, follicular thyroid cancer FTC-133 cell line, and a number of primary cultures from fresh thyroid tissue excisions. **Results:** NIS, TSHR, and Tg were detected by immunofluorescent staining in thyroid cancer tissue. Antibodies used for staining were isolated and sequenced for preparation of targeted CAR-T cell lines. Co-culturing of K-1 or FTC-133 cells with the anti-TSHR CAR-T showed a reduction of GFP intensity, indicating cell killing. Significant killing was confirmed with Caspase 3 and 7 assays. We observed 2.5 fold increase in killing in K-1 cells and 2.2 fold increase in killing in FTC-133 cells with anti-TSHR CAR-T cells compared to non-specific CAR-T cells. **Conclusion:** As well-differentiated thyroid cancer cells express at least three distinct extracellular protein motifs, targeting to thyroid tissue is achievable using the CAR-T cell construct to induce selective ablation of thyroid tissue. For patients with differentiated thyroid cancer refractory to traditional therapies, CAR-T presents an opportunity for a potentially efficacious new treatment option. Further studies in vivo are expected to document adverse effects and therapeutic index.

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Defining the Interplay Between EpCAM and EGFR in Cancer

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EpCAM is a transmembrane glycoprotein that is perhaps best known as the first tumor-associated antigen to be identified using monoclonal antibodies. Since its discovery, it has been well characterized as a homophilic, cell adhesion molecule that is that is expressed in normal epithelium and at even higher levels in epithelial cancers, including breast, pancreatic and lung cancers. EpCAM has been widely targeted by numerous groups in several different epithelial cancers. Even though numerous antibody-based and vaccine-based strategies targeting EpCAM have been investigated, the biological role and functional significance of EpCAM expression in cancer remains unclear. EpCAM expression appears to be associated with prognosis in several epithelial cancers, although in some cancers it is associated with a favorable prognosis (renal cell, rectal) and in others it is associated with a poor prognosis (lung, pancreatic, breast), a phenomenon that has been referred to as the "double-face" of EpCAM. Thus, the EpCAM biology is context-dependent. Preliminary screening of over 40 cancer cell lines showed that EpCAM was not only overexpressed in A431 cells, but also had the greatest impact on the invasive capability of this cancer. A431 is an epidermoid cell line known to overexpress EGFR and the model cell line for EGFR signaling and functional studies in the field. Cells overexpressing EpCAM enhanced the invasion and migration of EpCAM of A431 while cells lacking EpCAM did not, suggesting a role of EpCAM in the biology of EGFR-overexpressing cells. Further biochemical analysis indeed showed that lentiviral knockdown of EpCAM increased EGFR activity, assayed through increased phosphorylated EGFR via immunoblotting. Additional immunoprecipitation studies and Western analysis showed that EpCAM binds directly to activated EGFR. These data suggest a role for EpCAM in EGFR signaling. The Gillanders Laboratory has also shown that EpCAM expression is regulated by ERK both directly and indirectly and that specific ablation of EpCAM is associated with enhanced ERK activity. EpCAM regulation of ERK is through physical interaction; EpCAM binds to ERK in the cytoplasm. ERK also regulates EpCAM expression at the transcriptional level directly - by binding the EpCAM promoter, and indirectly - by activation of EMT-associated transcription factors such as Slug, Twist and Snail. These transcription factors are also critically involved in the regulation of EMT. These data demonstrate that EpCAM binds to and inhibits oncogenic proteins directly and that EpCAM cellular localization is dynamically regulated. Defining the mechanism(s) of EpCAM-dependent regulation of EGFR signaling in EGFR-amplified cancers such as lung and breast cancer may lead to new therapeutic strategies for EGFR inhibition in these cancers.

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Sex Differences in Obesity-Associated Vascular/Perivascular Remodeling and Adipokine Production in Obese Patients Undergoing Roux-en-Y Bariatric Surgery

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In human obesity, inflammation and adipokine production affect mortality and cardiovascular tissue remodeling independent of BMI. Studies measuring plasma adipokine levels suggest that anti-inflammatory adipokines including omentin-1, secreted frizzled-related protein 5 (SFRP5), and adiponectin improve cardiovascular function while pro-inflammatory adipokines, like resistin, impair cardiovascular function. Furthermore, adipokine production and function differ in males and females. Therefore, we tested the hypothesis that in human obesity, the degree of vascular/perivascular pathology is sex-dependent and associated with a distinct adipokine profile. **Methods**: Mesenteric vessels and perivascular adipose tissue were obtained from 26 obese patients (4 males, 22 females) undergoing Roux-en-Y bariatric surgery. A blinded semi-quantitative approach was used to assess 4 criteria: Fibrosis, adventitial wall thickening, angiogenesis, and immune cell infiltration. Samples with 2+ criteria were considered abnormal (pathology). Expression levels of adiponectin, omentin-1, resistin, and SFRP5 in perivascular fat (PVAT) were evaluated by qRT-PCR. All collected data were analyzed by histopathology and sex. **Results**: 60% of female tissue samples displayed significant pathology, which consistently involved fibrosis and adventitial wall thickening. Significant pathology was not observed in male tissues. When categorized by histopathology alone, females with pathology had 3-fold lower omentin-1 mRNA and 2-fold higher resistin mRNA vs. females and males without pathology. When compared to males, females without pathology had 10-fold higher omentin-1 mRNA whereas obese females with pathology had only 1.6-fold higher omentin-1 mRNA and 1.6-fold higher resistin mRNA. In females only, females with pathology had 5-fold lower omentin-1 mRNA, 2-fold higher resistin mRNA and 2-fold higher SFRP5 mRNA vs. females without pathology. None of these findings were associated with age, BMI, type II diabetes, dyslipidemia, or hypertension. **Conclusions:** In the mesentery, females are more susceptible to obesity-associated vascular/perivascular tissue remodeling than males. When compared to obese females without pathology, obese females with pathology have lower omentin-1 mRNA and higher mRNA levels of resistin and SFRP5. Therefore, omentin-1, resistin and SFRP5 may be useful adipokines in identifying obese females at risk of worsening cardiovascular tissue quality.

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Identification of Neuropeptides Differentially Expressed in Runner Versus Sedentary Mice by MALDI TOF-mass Spectrometry and Label-free Profiling and LC-MS-based Stable Isotopic Labeling in a Mouse Exercise Model

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One of the major obstacles for treating drug addiction is relapse, which often occurs as a conditioned response to drug-paired cues. Recently, it was discovered that incorporating exercise into the treatment plan can reduce relapse to cocaine abuse. A mouse model was developed which recapitulates accelerated extinction or abolished conditioned place preference for cocaine. However, the molecular and physiological changes in the brain that mediate cue-induced craving and how these changes are altered from exercise are poorly understood. The dentate gyrus of the hippocampus is a likely epicenter for exercise-contextual conditioning interactions, but the role of peptides, crucial signaling molecules in the region, is virtually unknown. Therefore, we conducted an experiment in which male C57BL/6J mice were conditioned to cocaine and then housed with or without access to running wheels for 28 days, followed by a CPP test. The following day after the CPP test, mice were placed into the texture where they either experienced cocaine or saline for 30 min. Mice were immediately euthanized after 30 min of context exposure. Brains were extracted, flash frozen, and brain punches from the dentate gyrus were analyzed using two different mass spectrometry (MS)-based quantification methods to detect peptide changes: label-free matrix-assisted laser desorption/ionization (MALDI) MS profiling and stable isotopic labeling with liquid chromatography (LC) and electrospray ionization (ESI) MS detection. Combined, the two analyses yielded 27 peptides that showed significant changes in amounts between runner and sedentary animals. Many of the peptides were derived from myelin basic protein, indicative of a change in the composition of the dentate gyrus of animals that exercised. However, no differences were detected between the animals exposed to the saline versus cocaine-paired textures within the running and sedentary groups or collapsed across them. Results further add to the rodent exercise-hippocampus literature by characterizing a novel set of peptide biomarkers responsive to exercise in the dentate gyrus.

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Data Driven Analysis of Robotic Surgical Performance and Training

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Teleoperated surgical robots can be used as motion capture devices for surgeons' motions intraoperatively, and thus produce high frequency data which can be used to infer performance and safety measures regarding surgical motions and surgeon skills. A modern data-driven approach to surgical motion analysis can provide improved abilities to track surgical trainees' progression along the long learning curve of surgical training. Real-time intraoperative feedback could also be designed from online data processing and detection of unsafe surgical motions or patterns indicating surgeon fatigue or distraction. Basic research is still required to enable such applications, regarding both the questions of analysis techniques for this type of data as well as regarding how data from such often-proprietary technologies can be leveraged in clinical practice. Meanwhile laboratory research is being performed on the Da Vinci Research Kit (DVRK) which enables open source engineering and free data collection in a laboratory setting. Experiments of robotic-assisted microsurgical anastomosis training under motion capture through the DVRK have been performed and represent a rich dataset for studying possible analysis techniques for surgical skill evaluation and progression tracking from surgical motion data. Support vector machines and other machine learning techniques are investigated for the analysis of this high dimensional surgical motion time series data.

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Disrupting Host Arginine-Associated Metabolic Pathways as a Potential Therapeutic Approach for Treating Ocular Herpes Simplex Virus Infections

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Worldwide, over 400 million people aged 15-49 years are estimated to be living with Herpes Simplex Virus (HSV)-type 2 and approximately 4 billion people aged 0–49 years had prevalent HSV-1 infection. HSV infection and reactivation is associated with a broad spectrum of disease presentations, including painful genital ulcers, meningoencephalitis, neonatal morbidity and mortality, as well as corneal blindness. As obligate intracellular parasites, viruses are reliant on host metabolism and macromolecular synthesis pathways. Of these biosynthetic processes, many viruses, including HSV, are absolutely dependent on the bioavailability of arginine, a non-essential amino acid that is critical for many physiological processes required for viral replication. Importantly, these arginine-associated metabolic processes also facilitate pathophysiological inflammation-associated progression of disease. We therefore hypothesized that therapeutics that could disrupt host arginine-associated metabolic pathways would simultaneously inhibit HSV replication and suppress inflammation-associated disease progression. In order to therapeutically modulate arginine levels, we developed and evaluated a biological therapeutic, human Arginase-I (hArgI), which depleted arginine by hydrolyzing it into ornithine and urea. The coding sequence of hArgI and a control red fluorescence protein (RFP) was optimized for bacterial codon usage and cloned into a T7-mediated bacterial expression pET vector. Protein expression was induced by IPTG and cell lysates were assessed for expression and solubility. Soluble HIS-tagged protein products were column purified and analyzed for protein purity, concentration, and the specificity for the final products. The dependence of HSV-1 on arginine levels to facilitate viral replication, spread and transmission was assessed on Vero cells maintained in media with various arginine concentrations. HSV-1 replication, transmission and cell-to-cell spread exhibited a marked and dose-dependent decrease in its ability to replicate as arginine concentrations were limited. hArg1 was shown to be able to deplete extracellular arginine levels *in vitro* leading to decreased HSV-1 replication. *In vivo* hArg1 was shown to decrease both viral and immune-mediated disease in rabbit models of herpetic eye disease. Our results show that both viral replication and host-inflammatory processes are absolutely dependent on extracellular arginine levels and that hArgI can quickly and effectively deplete extracellular arginine pools. Therefore, targeting host arginine-associated metabolic pathways is an effective means of controlling the viral replicative processes of HSV as well as simultaneously disrupting inflammation-associated disease processes. Further exploration into the breadth of viruses inhibited by targeting host arginine catabolism, as well as the ability to suppress other inflammation-associated pathophysiological disease processes is warranted.

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Circadian Dysregulation in Colon Cancer Cells: ERK-dependent Increased and Aberrant TIMELESS Expression Promotes Colon Cancer Cell Survival

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Multiple studies have revealed that Ras-driven tumors acquire vulnerabilities by adapting cellular mechanisms that promote uncontrolled proliferation and suppress apoptosis. Targeting these vulnerabilities provide opportunities to develop novel, efficacious cancer therapeutics that lack the side effects accompanying current therapies. Kinase Suppressor of Ras 1 (KSR1), a molecular scaffold for the Raf/MEK/ERK kinase cascade, represents one of these vulnerabilities as the *ksr1*$^{-/-}$ mice are developmentally and phenotypically normal, but are resistant to Ras-driven tumor formation. RNA interference (RNAi) of KSR1 selectively kills malignant, colon cancer cells, but not immortalized, non-transformed human colon epithelial cells (HCECs). To identify additional vulnerabilities and novel therapeutic targets in cancer cells that are required for cancer cell survival but not normal cell survival similar to KSR1, we used a gene expression-based signature screening approach termed Functional Signature Ontology (FUSION) to screen 15,172 genes in the K-Ras^{G13D}-bearing human colorectal cancer cell line HCT116. Using KSR1 as a positive control, we quantified the functional similarity between KSR1 and each individual gene screened using Euclidean Distance and Pearson Correlation similarity metrics. Hits were further prioritized using bioinformatic analysis. TIMELESS was identified as a target and found to be upregulated at both the mRNA and protein level in seven colon cancer cell lines compared to HCECs (~4 fold, $p < 0.0001$). Additionally, in human colon tumors, TIMELESS mRNA is increased compared to normal colon tissue (~2.2 fold, $p < 0.0001$)(TCGA). In HCECs, but not cancer cells, TIMELESS

displays a circadian expression following cell synchronization by serum shock. Treatment with the ERK inhibitor SCH772984 robustly decreases TIMELESS expression and partially restores circadian expression of TIMELESS. This effect is specific to ERK as ERK depletion by RNAi or upstream inhibition of MEK by PD0325901 recapitulates the effects. Adding mutant Ras^{G12V} to HCECs robustly increases the expression of TIMELESS, which can be abrogated with the addition of SCH772984. This demonstrates the aberrant TIMELESS expression is dependent upon increased Ras signaling in cancer cells. Importantly, TIMELESS depletion decreases the number of viable colon cancer cells by 49% in HCT116s and 37% in Caco2 cells ($p < 0.0001$, N=6), but does not affect viability or proliferation in HCECs. This suggests that Ras-driven tumors upregulate TIMELESS to support their survival and proliferation by dysregulating circadian cycles to release constraints on proliferation as circadian rhythm and cell cycle are inextricably linked. Our data indicate that FUSION provides a platform for identifying novel therapeutic targets and demonstrates the potential to identify oncogene-specific vulnerabilities in an unbiased manner. The increased and aberrant expression of TIMELESS represents a unique vulnerability in Ras-driven tumors and reveals a novel mechanism cancer cells employ to circumvent normal proliferative constraints through circadian dysregulation. This suggests restoring normal circadian rhythm may be an efficacious approach in the treatment of cancer.

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TGF-β Induced *De Novo* Serine Synthesis Is Required For Collagen Production

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Idiopathic pulmonary fibrosis (IPF) is a progressive disease with a median survival of 3.5 years. IPF is characterized by increased TGF-β, the key cytokine in IPF, which induces deposition of excess collagen by fibroblasts via SMAD signaling. Collagen is unique due to it high glycine content (33%). Glycine is a non-essential amino acid synthesized from serine, synthesis of which diverges from glycolysis via a glycolytic intermediate 3-phosphoglycerate in a three-step enzymatic reaction starting with phosphoglycerate dehydrogenase (PHGDH). Serine then fuels glycine synthesis via serine hydroxymethyltransferase 1 (SHMT1) or 2 (SHMT2), which are compartmentalized in the cytoplasm and mitochondria, respectively. This study aimed to investigate whether TGF-β is responsible for increased glycolysis and that increased glycolysis promotes *de novo* serine and glycine synthesis. **Methods:** Human lung fibroblasts (HLFs) were treated with TGFβ (1 ng/mL) for up to 48 hours before we measured glycolytic rates (Seahorse XF^e24), the mRNA (qPCR) and protein (western blotting) expression of enzymes of glycolysis and *de novo* serine and glycine biosynthesis pathway (PHGDH, PSAT1, and SHMT1/2). To evaluate the effect of glycolysis HLFs were treated in the presence 2deoxy-D-glucose, and sodium oxamate or galactose. We also measured protein (western blotting) expression of collagen, and phosphorylation of SMAD3 and mRNA (qPCR) expression of TGF-β target gene expression including collagen, αsmooth muscle actin (SMA), and connective tissue growth factor (CTGF). To evaluate the effect of *de novo* serine and glycine biosynthesis pathway on collagen production, HLFs expressing control or siRNAs targeting PHGDH, SHMT1 and SHMT2 were treated with TGF-β in the presence or absence of serine and glycine in media. In addition, HLFs were treated with PHGDH enzyme inhibitor, CBR5884. Using ^{12}C or ^{13}C-Glucose, we also performed proteomics to assess the incorporation of glucose-derived glycine into and collagen. Western blotting and immunohistochemistry staining for PHGDH and SHMT2 was performed in IPF lungs. **Results:** TGF-β induced glycolysis, which was required for collagen and SMA protein production without affecting gene expression or SMAD phosphorylation. TGF-β induced *de novo* serine and glycine pathway enzymes except SHMT1. Both pharmacologic inhibition of PHGDH and genetic deletion of PHGDH and SHMT2 attenuated collagen production. Metabolic labeling experiments showed incorporation of labeled glucose into collagen. Lungs from patients with IPF demonstrated increased expression of PHGDH and SHMT2. **Conclusions:** TGF-β promotes glycolysis to promote *de novo* serine biosynthesis, which is required for glycine and collagen protein synthesis in fibroblasts.

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Percutaneous Balloon Compression as Treatment to Trigeminal Neuralgia: 14 Years of Experience in a Single Center

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Percutaneous balloon compression of the Gasserian ganglionic has been used to treat Trigeminal Neuralgia (TN) since 1983. **Methods**: We performed a retrospective study on 222 patient's records which have received 242 procedures of Percutaneous Balloon Compression (PBC) as treatment for TN. A 6 months follow-up period after surgery was needed to be included in the study. They were operated at Functional Neurosurgery Unit in Brazil from February 2002 to July 2016. **Results:** The patient's age ranged from 29 to 91 years (mean, 62, 2 years), 43% were males and 57% were females. Rare cases of bilateral trigeminal neuralgia were seen in 5 patients. Immediately after surgery, 193 (79,7%) patients became pain-free and 34 (14%) patients became pain free during the following 4 days. Carbamazepine was suspended in 93.7% of cases. Out of the total, only 13 (5,37%) patients related residual pain on follow-up. Hypoesthesia was reported after 83,8% of procedures. In addition, bradycardia was seen in 58,1% of cases during foramen ovale puncture. Transitory complications such as diplopia (2,47%), otalgia (1,23%) and tinnitus (1,23%) were reported. Regarding to balloon appearance, pear and dumb-bell shapes were detected in 74,8% and 7,4% of procedures, respectively. Only 15 (6,19%) patients needed a reoperation due to pain persistence. **Conclusion**: PBC showed to be an effective and safe technique which provides high rates of pain relief (93,7%) in the following 6 months after surgery. The Carbamazepine's use was markedly reduced (93,7%). In addition, relapse of pain occurred in few cases (5,37%). Complications were minor and transitory. Comparing statistically, we can stat that the balloon shapes (82,2%) and hypoesthesia (83,8%) findings had a positive influence on pain relief rate (93,7%). Our findings support that PBC should be considered as primary surgical treatment of trigeminal neuralgia.

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Bacteriophage in the Human Gastrointestinal Tract and Association with Infection

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Bacteriophages within the gastrointestinal (GI) tract represent a diverse population and may have a role in human health. In the present study, we sought to sequence the bacteriophage metagenome (phageome) and isolate phages that can inhibit enteric pathogens. Fecal samples were collected, and the supernatant was filtered to recover phage communities from people with enteric infections caused by *Salmonella*, *Shigella*, *Escherichia*, and *Campylobacter* as well as otherwise healthy individuals (controls). Phages from each phage community were titered and used to infect three different enteric pathogens, *Salmonella*, *Shigella*, *Escherichia*, and two strains of commensal *Escherichia coli* (*E. coli*). The phageome was sequenced and assembled from each sample and individual phages were isolated following pathogen lysis. Phage communities were significantly more likely to have a bactericidal effect against the three enteric pathogens when compared to commensal *E. coli* (p=0.0001). Moreover, phage communities from healthy individuals were more likely to lyse the three pathogens than the phage communities recovered from patients with an acute infection (p=0.0333). A total of 15 distinct phageome profiles were found and were unique to each individual. Principle Coordinate Analysis demonstrated that these phageome profiles clustered based on the health of the individual. *Salmonella phage 100268_sal2*, was found only in fecal samples of patients with acute *Salmonella* infections, while *Bacillus phage* was conserved across all samples. Isolation and sequencing of a single phage capable of lysing multiple strains of Shiga toxin-producing *E. coli* revealed a novel phage with > 95% identity to a subset of phages in the T5-like virus family of *Siphoviridae*. Retrospective analysis of phageomes detected a member of this subset of T5-like virus family in 93% of the 15 human fecal samples examined to date. These results suggest that there is considerable variation in both the function and composition of the phageome of the GI tract from patients with and without enteric infections. A further assessment of the phageome in more individuals is therefore warranted and may enable the identification and use of phages as therapeutics to treat or prevent enteric disease.

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The Airway Surface Liquid of Large and Small Airways Have Different Antimicrobial Activity

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The native airways have a variety of host defense mechanisms against respiratory tract infections. One of the first is the airway surface liquid (ASL), which contains antimicrobial peptides (AMPs). Decreased ASL antimicrobial activity has been implicated in the pathogenesis of several airway diseases. Additionally pneumonias generally originate in the small airways. We hypothesize that the antimicrobial activity of ASL in the small airways is less than that of the large airways. **Methods:** Using microdissection, we isolated the epithelia of large and small porcine airways. We selected this animal model due to the fact that it has been shown to exhibit many human lung diseases. Primary airway epithelial cells were cultured at the air-surface interface. The ASL was extracted by washing the cells with 50µL of 10mM sodium phosphate solution, pH 7.4. ASL was then challenged with ~$5.76x10^4$ colony forming units (CFUs) of bioluminescent *Staphylococcus aureus* (*XEN29)* in an opaque 96-well plate. After 10 minutes of exposure the relative light units (527nm) were measured. In order to validate the luminescent data, ASL was challenged with ~1000 CFUs of *XEN29*. After 10 minutes of exposure we plated the remaining bacteria and counted CFUs after overnight incubation at 37°C. **Results**: We found a trend towards increased bacterial luminescence in the ASL from small airways compared to large airways 10 minutes after bacterial challenge. In addition, this result was consistent with the number of CFUs after a bacterial challenge. **Conclusion**: We conclude that the ASL from the small airways has decreased antimicrobial activity compared to the large airways. This may be a reflection of the different environments to which they are exposed to on their apical surface. It is also known that variables such as pH and ionic strength alter the activity of antimicrobial peptides in the ASL. However in this experiment both the pH and ionic strength do not differ between groups. Further studies will concentrate on other mechanisms that may explain the difference between the antimicrobial activity of large and small airway ASL.

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Restoration of HCV-specific CD4 T Cell Function in the Postpartum Period

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Hepatitis C virus is a leading cause of advanced liver disease around the world. Infection becomes persistent in the majority of those infected due to impaired T cell immunity. Exhausted HCV-specific T cells lose the ability to proliferate, lose cytotoxic activity, demonstrate impaired cytokine secretion, and may be deleted. Exhaustion persists in chronic hepatitis C, even after cure by direct acting antivirals. An exception to this rule, however, is found in relation to pregnancy. By 3 months postpartum, one-third of chronically infected women regain control over HCV replication, with declines in HCV viremia exceeding 1 $\log_{10}$ and in some cases approaching 5 $\log_{10}$. Antiviral T cell activity has been implicated in acute and, more recently, postpartum control of HCV, but it is still unclear whether CD4 T helper (Th) or cytotoxic CD8 (Tc) T cells are the primary drivers of this response. We hypothesize that HCV-specific CD8 T cells regain function in chronically infected women in association with postpartum viral control, under modulation by some unknown factor related to pregnancy and delivery. **Methods:** Here we have performed analyses of HCV-specific T cell function by intracellular cytokine staining in order to understand the relative roles of CD4 and CD8 T cells, as well as various Th and Tc subsets in improved postpartum control of chronic hepatitis C. **Results:** Contrary to our hypothesis, HCV-specific CD8 T cell function did not distinguish women with postpartum viral control from those without. However, we have shown that there is a surprising restoration of HCV-specific Th1 cytokine secretion in the postpartum period that associates with viral control. In the postpartum period, controllers demonstrate

significantly higher frequencies of HCV-specific CD4 T cells secreting IL2 alone or co-secreting IL2 and IFNγ (p= 0.0064 and p= 0.0024). **Discussion:** Improvement in the function of exhausted CD4 T cell responses in the postpartum period suggests that alteration of some factor during this time allows HCV-specific CD4 T cells to recover their ability to secrete cytokines and to promote antiviral immunity. This process occurs without treatment, and our findings suggest that T cell exhaustion is a naturally reversible process *in vivo*. Identifying the factors that promote postpartum CD4 T cell recovery should be of high priority, as modulation of these factors could potentially be used to bolster vaccines or with antivirals to promote T cell recovery and protective immunity following the cure of infection.

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Synchronous Inverted Papilloma and Recurrent Respiratory Papillomatosis: Case Report and Review of the Literature

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The pathogenesis of recurrent respiratory papillomatosis (RRP) and inverted papilloma (IP) are both associated with the Human Papillomavirus (HPV). This relationship is less clearly defined for IP than for RRP. We report a case of histopathologically proven IP occurring in a patient with laryngeal RRP, with the same strain of HPV confirmed in both sites. This represents the first report of IP occurring within RRP. A review of the literature was conducted to better understand the incidence of RRP in the nasal cavity and the role of HPV in the pathogenesis. **Methods**: Ovid Medline and PubMed databases search was conducted using the following keywords: "inverted papilloma", "recurrent respiratory papillomatosis", "Human Papilloma Virus", and "sinonasal". Reference lists were reviewed by all authors for relevance. Articles reporting IP in sites other than the sinonasal cavity were excluded. **Results:** No cases of concomitant RRP and sinonasal IP were identified. No cases of sinonasal RRP were identified. Two cases of diffuse sinonasal papillomatosis without history of RRP were identified. We reviewed the data on the role of HPV in the pathogenesis of IP and found conflicting data. Previously published studies report HPV involvement in 0-75% of cases. The type-specific detection method was identified as a potential reason for the variability in studies. **Conclusions**: The exact role of HPV in the pathogenesis of IP continues to be debated. Recent isolated studies have shown a higher correlation between histopathologically proven specimens of IP and HPV. This case adds support for the role of HPV in the pathogenesis of IP.

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Measurement of αβ T Cell Receptor Mispairing for Selection of Effective Gene Therapy TCRs

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The heterodimeric α/β T cell receptor (TCR) is the sole determinant of T cell specificity. In TCR gene therapy, patient T cells are transduced with tumor-specific α and β TCR genes to impart anti-tumor immunity. However, introduced TCR α and β chains can mispair with the transduced T cell's endogenous TCR β and α chains, respectively, reducing the number of tumor-specific TCRs on the surface and potentially generating autoreactive TCRs. So far, a TCR's propensity to mispair has been an undefined property. We are developing a quantitative assay to measure the extent to which mispairing occurs for TCRs of clinical interest. We identified two antibodies that bind the constant portions of the endogenous TCR α and β chains. These epitope sites were mutated in the transduced TCR chains and synthetic epitope tags were added to the N-terminus of these chains to enable their orthogonal recognition by a second set of two antibodies. Using a sandwich ELISA, we capture the transduced α or β TCR chain with a tag-specific antibody and then detect the β or α chain to which each is paired using a second antibody. Signal is observed only when both capture and detection antibody targets are present. Therefore, all possible α/β heterodimers can be distinguished by using different antibody pairs and each heterodimer can be quantified by comparison to a standard curve. Although there were no clear differences in the mispairing ratios of TCRs within a cell, there seems to be a stratification of mispairing ratios for TCRs only expressed on the surface. Since the assay focuses on ratios, the variability is not due to TCR strength (how well a TCR is expressed on the surface), but rather an inherent property of TCRs that determines whether a mispaired heterodimer will be expressed. Future experiments will hopefully validate our results.

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Targeting MYC Oncogene to Overcome Chemoresistance in a Novel Preclinical Model of Muscle Invasive Bladder Cancer

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Bladder cancer (BCa) is the fifth most common cancer in the United States. The basal subtype muscle invasive bladder cancer (MIBC) has the shortest survival of approximately 25 months due to the onset of chemoresistance and associated toxicity. Therefore, there is a clinical need to understand how to sensitize bladder tumors to acceptable levels of chemotherapy and determine a novel treatment that will be effectively treat chemoresistant BCa. While human BCa cell lines exist, they may not reflect the complexity of human BCa and adaptation to treatments due to divergent evolution during the long-term in-vitro culture. To overcome this challenge, a novel mouse derived allograft (MDA) of MIBC resistant to cisplatin (CDDP), the most commonly used chemotherapy drug for BCa, was developed using a carcinogen, OH-BBN (0.1%, N-Butyl-N-4-hydroxybutyl nitrosamine). OH-BBN induced BCa was confirmed to represent basal subtype MIBC, which overexpresses the transcription factors, p63 and MYC. When the MDA was treated with CDDP, there was chemoresistance showing no significant decrease in the tumor growth rate. Only at a very high and clinically unacceptable dosage, there was a significant decrease in the tumor growth rate, however, p63 and MYC were still overexpressed. Furthermore, a patient derived xenograft (PDX) from MIBC patients who are experiencing chemoresistance was also adapted by implanting their BCa tumors into mice. The clinical samples have been confirmed to overexpress p63 and MYC. As seen in both the MDA and PDX models, transcription factors are overexpressed in cancer cells. Transcription factors, which drive gene signatures associated with normal organ development as well as aggressive cancer phenotypes, may be the key determinants of progression and therapeutic response. In the normal mouse genitourinary tract, p63-positive cells are necessary for the development of bladder and are capable of forming all cell

lineages. This suggests that p63 may serve an equally important role in the basal MIBC etiologies. Targets of p63 with well-established oncogenic function include MYC, which is directly regulated by p63. MYC signaling, in other cancer types in which CDDP is commonly used, has been identified as a possible cause of resistance and a plausible therapeutic target. Since direct pharmacological targeting of p63 and MYC is challenging, an established bromodomain inhibitor (BETi), JQ1, has been used to inhibit MYC expression and function. JQ1 and CDDP were tested individually and in combination on the MDA and PDX. The average tumor volumes were compared, and results showed in both models that the effects of the combination treatment was significant ($p < 0.05$). This suggests that the p63 signaling pathway drives MYC signaling to regulate progression and chemoresistance in MIBC. In the future, the combination treatment in an orthotopic mouse model can be investigated.

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A Murine Model of a Ubiquitous Human Herpesvirus Causes T-cell Depletion

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Herein, we describe a novel murine betaherpesvirus called murine roseolovirus (MRV) related to murine herpesvirus 3 (Mouse Thymic Virus [MTV]). MRV causes severe thymic necrosis in neonatal mice, characterized by a loss of $CD4^+$ T-cells in the thymus and spleen. Infected neonatal mice reproducibly recover from infection replenishing normal complements of $CD4^+$ cells in both the thymus and periphery. We also demonstrate direct infection of $CD4^+CD8^-$, $CD4^+$ $CD8^+$, $CD4^-$ $CD8^+$ and $CD4^-CD8^-$ cells from infected thymi. The identity of this virus, its relationships to other viruses, and the mechanisms by which it induced the underlying pathology remain incompletely understood. We sequenced the virus and found it was indeed a double-stranded DNA virus with genome structure consistent with classification as a betaherpesvirus. Phylogenetic analyses show MRV is most closely related to human roseoloviruses, hence its name. We detailed dynamics of infection showing viral genome copies peaked in infected thymi, although MRV also was present in other organs. Finally, we investigate the mechanisms of pathogenesis and immunity to MRV. Our studies identify the first murine homolog for the human roseoloviruses (human herpesvirus 6A, 6B and 7), which infect nearly 100% of the worldwide population. Many biological and clinical ramifications of roseolovirus infection in humans have been hypothesized, but studies showing definitive causative relationships between infection and disease susceptibility are lacking. Here we show that MRV infects the thymus and causes T cell depletion, suggesting that other roseoloviruses may have similar properties.

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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. Four variants that were shared across ancestries from the “credible sets” of variants accounting for 99% of the posterior probability in any single ancestry were identified as the Ancestrally Informed Credible Set (AICS). The results replicated in an independent set of trans-ancestral cases and controls. We 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. Finally, to assess whether these variants show genotype-dependent enhancer activity, we generated luciferase reporter constructs and identified genotype-dependent repressor activity. 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 multiple genetic variants in a lupus-risk haplotype that is shared across ancestries. One of these variants further demonstrates genotype-dependent regulatory activity In B cells.
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Endoplasmic Reticulum Aminopeptidase 1 Mediates Risk for Spinal Ankylosis and Osteoporosis

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Ankylosing spondylitis (AS) is a debilitating chronic inflammatory disease characterized by fusion of the spine and sacroiliac joints. AS patients are prone to osteoporosis of the trabecular bone of the vertebral bodies, making them susceptible to spinal fractures and associated neurological defects, contributing significantly to the morbidities associated with AS. Genome-wide association studies (GWAS) of AS patients identified multiple polymorphisms in Endoplasmic Reticulum Aminopeptidase 1 (ERAP1) which are associated with development of AS. Previously, we have shown that ERAP1 is an important regulator of innate immune responses in mice and human immune cells. Without ERAP1, mice generate an exaggerated inflammatory response to multiple innate stimuli, including increased levels of TNFα and IL-6 in serum, which are also elevated in AS patients. Because TNFα and IL-6 cytokines play an important role in bone homeostasis, we hypothesized that ERAP1 functions may also play a role in the skeletal system. **Methods:** μCT imaging was used to evaluate bone morphology and density of the axial skeleton in WT and ERAP1-/- mice over their lifetimes. Bone marrow cells were isolated from 25-week-old WT and ERAP-/- male mice and cultured in vitro for osteoclastogenesis and functional assays: osteoclast numbers were quantified using TRAP staining and mean resorption pit sizes were assessed by culturing on dentin disks, respectively. Marrow cells were also cultured for osteoblastogenesis quantitation by alkaline phosphatase staining. Static and dynamic histomorphometry analyses were used to assess osteoblast counts and bone formation rate, respectively. **Results:** Unlike WT mice, ERAP1-/- animals developed spontaneous fusion of the lumbar vertebra 6 (L6) with sacrum (seen in 30/35 mice), mimicking the hallmark feature of spinal ankylosis in AS patients. Interestingly, reduced trabecular bone volume fraction was observed in the L6 and sacrum of ERAP1-/- mice starting at 14 weeks of age. Primary bone marrow cultures demonstrated that ERAP1-/- mice display increased osteoclastogenesis and osteoclast activity, findings consistent with the osteoporotic phenotype observed in these same animals. While, we detected increased osteoblastogenesis, indicating higher potential of ERAP1-/- bone marrow cells to become osteoblasts; total number of osteoblasts per trabecular surface area in the sacrum was significantly lower in ERAP-/- animals compared to WT. Dynamic histomorphometry analysis showed no difference in bone formation rate between WT and ERAP-/- mice in their sacrum, indicating no overall difference in the osteoblast activity in this region. **Discussion**: We identified a novel animal model phenocopying axial bone ankylosis, which is the hallmark feature of AS. ERAP1-/- mice demonstrate spinal fusion and decreased bone density in the same anatomic regions. Enhanced responses to innate stimulation and skeletal phenotype observed in ERAP1-/- mice, make them a suitable model for studying pathogenesis of AS and testing the efficacy of therapeutic agents for the multiple features of this disease.

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Computational Reconstruction of 3-D Chromatin Structure of a Super Enhancer Locus at 11 nm Resolution

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Using Hi-C data, we reconstruct the three-dimensional structure of a 330 KB contact domain encompassing an 80 KB super enhancer locus within the K562 cell line. Using a novel Monte Carlo sampling technique, we are able to generate an ensemble of chromatin conformations at 11 nm resolution. We then analyze the simulated contacts for non-trivial interactions and genomic regulatory significance.

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Genotoxic Effects of PCB 153 Cause NF-κB Dependent Increases in Intestinal Permeability and Intestinal Inflammation Via the ATM/NEMO Pathway

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Xenobiotics are a cause of inflammation and have been hypothesized as a reason for the increase in inflammatory bowel diseases. Polychlorinated biphenyls (PCBs) are ubiquitous and persistent organic pollutants that adversely affect human health and the immune system. PCBs are highly lipophilic which enables them to bio-accumulate in food chains leading to high levels in organisms important for human consumption. Despite dietary exposure being one of the main routes of exposure to PCBs, the human gastrointestinal tract has been widely ignored when studying the proinflammatory effects of PCBs. We investigated the effects of PCB 153, the most common PCB congener found in the environment, on the gut to elucidate whether it leads to intestinal inflammation. We then investigated the mechanism of its inflammatory effects and its effects on gut permeability. Mice were exposed via oral gavage to PCB 153 and intestinal epithelial cells (IECs) were collected and evaluated for genotoxicity and inflammation. Gut permeability was also assessed. Human intestinal epithelial cells (SW480) were exposed to PCB 153. NF-κB activation was measured using a reporter assay and downstream consequences were assessed. Mice exposed to PCB 153 had increased gut permeability and an increase in inflammatory cytokine expression in their IECs. This inflammation occurred concurrently with genotoxic damage and NF-κB activation in the IECs. Inhibition of NF-κB ameliorated the increase in intestinal permeability. Exposure of SW480 cells to PCB 153 led to an increase in inflammatory cytokine expression and an increase in NF-κB activity. This activity was triggered by genotoxic effects of PCB 153 and the subsequent activation of the ATM/NEMO pathway. These results suggest that oral exposure to PCB 153 is genotoxic to IECs and that this induces downstream inflammation in the intestinal epithelium via NF-κB activation through the ATM/NEMO pathway. Activation of NF-κB is also responsible for the increases in intestinal permeability.

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Identification and Characterization of Novel Oncogene Candidates in Invasive Breast Carcinoma

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Invasive breast carcinoma (IBC) is the most common cancer in women worldwide, and certain subtypes of IBC remain incurable and carry a poor prognosis. The expression of oncogenes drives tumor behavior and defines subtypes of breast cancer that may be susceptible to targeted therapies (e.g. Trastuzumab for ERBB2-positive breast cancer). Oncogenes have increased mRNA expression in cancerous tissue relative to healthy tissue and, traditionally, have been identified through statistical testing for differences in average mRNA expression values between matched sets of normal and cancerous tissue. While useful for small sample sizes, these tests (e.g. Student's t-test) are limited in their ability to identify tumor subgroups that may be under common control of a specific oncogene. Previous studies have investigated the prevalence of genes with distinct two-component clusters of mRNA expression from hundreds of ovarian and breast tumors and found these clusters to be predictive of clinical outcomes such as disease-free survival time. Identifying genes with tumor-specific modes of mRNA expression that are elevated above a baseline in normal breast tissue represents an alternative approach to discover new oncogene candidates. Working with non-normally distributed data requires novel statistical approaches, therefore a new mixture model-based method was developed to identify oncogene candidates. Using RNA-sequencing data from 113 patients in The Cancer Genome Atlas with paired IBC and matched normal tissue, we uncover 11 oncogene candidates, each with a distinct cluster of low and high mRNA expression across tumors, but with uniformly low mRNA expression in normal tissue. To further investigate possible mechanisms behind the distinct mRNA expression clusters, we explore associations between DNA methylation, copy number variation, and the mode of mRNA expression for the 11 oncogene candidates using coefficient-penalized logistic regression, and discover that DNA methylation within the introns of genes is a primary driver of mRNA expression in a majority of these genes. Furthermore, we discover that decreased DNA methylation at enhancer regions present in a breast cancer cell line but not in a non-oncogenic mammary epithelial cell line predicts increased oncogene candidate expression. We propose a quantitative model whereby oncogene expression in breast carcinoma is regulated by a switch-like mechanism governed by DNA methylation at genomic regions with labile chromatin. The discovery of novel oncogene candidates in large cohorts can uncover previously unappreciated tumor subgroups that cluster together based on similarities in mRNA expression of individual oncogene candidates. These oncogene candidates – along with their mechanisms of expression – can be further investigated as therapeutic targets or prognostic biomarkers for the subgroup-specific treatment of incurable IBC.

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Cyclic di-GMP Interacts With Enteric Glia STING to Produce Type I Interferons and Innate Immune Response in the Enteric Nervous System

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Cyclic diguanylate monophosphate (c-di-GMP) is a bacterial second messenger molecule in environment-based regulation, important in the virulence of enteropathogens such as *Escherichia coli* O157:H7, *Vibrio cholerae*, and *Clostridium difficile*. C-di-GMP incurs innate immune responses, with dendritic cell activation in human cells and monocyte recruitment in murine models, prompting proinflammatory cytokine and chemokine release, and subsequent increase in adaptive immunity T cell response. The transmembrane protein stimulator or interferon genes (STING, also known as ERIS, MITA, MPYS, TMEM173) is the primary pathway for mammalian immune recognition of c-di-GMP and other cytosolic DNA; STING activation leads to downstream upregulation of type I interferons (IFNs). The enteric nervous system (ENS) controls gut reflexes, and inflammation-driven neuroplasticity contributes to the pathophysiology of gastrointestinal (GI) motility disorders, including inflammatory bowel syndrome (IBS) and inflammatory bowel diseases (IBDs). The proinflammatory response in GI motility disorders is primarily driven by increased innate immune activation, yet how the constituent cells of the ENS (neurons and glia) contribute to this process is not clear. Enteric glia express toll-like receptors (TLRs), major histocompatibility complex II (MHC II), and are capable of responding to bacteria; they have the potential to behave as antigen presenting cells and respond to cytokine release, but the details of these interactions and the possible downstream effects on ENS health are still unknown. Here, we tested the hypothesis c-di-GMP derived from enteric pathogens influences the neuronal control of gut functions through actions on enteric glia. Utilizing immunohistochemistry (IHC), our results show that enteric glia are the primary site of STING expression in the ENS. With 1mM c-di-GMP addition to ENS tissue and subsequent IFN-β ELISA and IHC for proinflammatory markers, we propose that upon c-di-GMP exposure, enteric glia utilize the STING pathway to produce type I IFNs and an innate immune response that contributes to inflammation and cell death. Ussing chamber measurements of c-di-GMP transportation demonstrate c-di-GMP would be able to cross the intestinal mucosa and lead to these effects. However, this is not seen *in vivo* administration of the compound through drinking water (~100 nmol/mouse/day) or enema (10 umol/mouse), as demonstrated by pellet production, colon macroscopic and histologic scoring, and IHC for immune response (gliosis, macrophage or neutrophil recruitment). In these cases, likely inadequate levels of c-di-GMP reach the ENS. In summary, c-di-GMP has the potential to act on enteric glial cells and cause subsequent ENS changes, but only under appropriate conditions.

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Diminished Rad50 Expression in Stem Cells from Human Uterine Fibroids Compared to Adjacent Myometrium Leads to Decreased DNA Repair Capacity, Compromised Genomic Integrity, and Increased Uterine Fibroid Tumorigenesis

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Although uterine fibroids (UFs), benign myometrial tumors, severely impact women's reproductive health and general well-being, the mechanism by which they arise has yet to be determined. Somatic mutations in *MED12* gene, detected in ~85% of sporadic human UFs, are currently thought to arise in myometrial stem cells (MSCs) converting them into UF tumor-initiating cells. Because defective DNA repair increases the risk of somatic mutation in these cells, they increase the risk of tumor development and growth. This suggests that additional mutations arising in fibroid stem cells (FSCs), unable to be repaired properly due to decreased RAD50 expression and, potentially, functionality, impair the FSCs' DNA repair capacity, causing further tumor growth and development. We aimed to analyze and compare the DNA repair system in the human Stro1+/CD44+ MSC and FSC populations isolated from patients affected by UFs.
Methods: Human fibroid (F) and adjacent myometrial (MyoF) tissues from reproductive age women (N=4) undergoing hysterectomy for treatment of UF were enzymatically digested to obtain single-cell suspensions. Magnetic beads were used to select MyoF and F cells positive for both Stro-1 and CD44 surface markers (MyoF+/+ and F+/+, respectively). Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed on F+/+ and MyoF+/+ cells to determine expression of *RAD50,* a gene pivotal to cellular DNA repair and the homologous recombination (HR) pathway. RAD50 protein expression was analyzed by Western blot. Immunocytochemical (ICC) staining for γ-H2AX and RAD50 was completed to detect DNA double-strand breaks (DSBs) by immunofluorescence (IF) analysis in cells with or without bleomycin-induced DSBs to determine rates of DNA repair. **Results**: By qRT-PCR and Western blot, F+/+ cells from all human samples demonstrated significantly ($p < 0.05$) decreased expression of *RAD50* and RAD50, respectively, compared to MyoF+/+ control cells isolated from adjacent tissue. In addition, F+/+ cells demonstrated increased γ-H2AX expression and slowed recruitment of RAD50 to DSBs vs. adjacent MyoF+/+ control cells. RAD50 is important in mammalian cells for MRE11-RAD50-NBS1 recruitment to sites of DNA DSBs via homologous recombination (HR) or non-homologous end-joining (NHEJ) pathways, which protect against tumorigenesis, to repair DNA DSBs. Its importance in HR suggests impaired RAD50 expression and function may be involved in UF development. Further characterization of this and other DNA repair-related genes in human F+/+ and MyoF+/+ cells is currently underway in our laboratory.
Conclusion: Our data suggest that decreased RAD50 expression and impaired DNA repair capacity, specifically of DNA double-strand breaks, in human fibroid stem cells contributes to UF development. Further studies are needed to reveal early changes in myometrial and/or fibroid stem cells leading to uterine fibroid development, and its possible contribution to the ethnic disparity of this disease.
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Muscle-enriched Circulating MicroRNAs miR-1 and miR-133a Display Dose-dependent Responses to Varying Intensities of Acute Aerobic Exercise: Implications for Developing Biomarkers of Cardiovascular Fitness

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As the popularity of exercise training in health and disease increases, so too does the importance of understanding the molecular mechanisms underlying exercise adaptation. Circulating microRNAs (c-miRNAs) – small, noncoding RNA molecules found in mammalian plasma that regulate gene expression post-transcriptionally – have been shown to mediate processes inherent in physiological exercise adaptation, including angiogenesis, inflammation, mitochondrial metabolism, cardiac and skeletal contractile force generation, and tissue hypertrophy. Although studies have demonstrated that specific c-miRNAs are upregulated in response to acute and sustained aerobic exercise training, it is unclear whether the response depends on exercise intensity. We hypothesize that the intensity of aerobic exercise impacts the response of muscle-derived c-miRNAs known to regulate cellular processes inherent to exercise adaptation.
Methods: Following initial cardiopulmonary exercise testing (peak V_{O2}: 61.7 ± 4.81 ml $kg^{-1} min^{-1}$), healthy male volunteers (N = 12; mean [SD] age: 20.77 [0.64]) ran once a week over four weeks for 30 minutes on a treadmill at 6 mph, 7 mph, 8 mph, and a peak pace in a randomized order. Using quantitative reverse transcription polymerase chain reaction, concentrations of plasma c-miRNAs enriched in muscle (miR-1, miR-133a, miR-206, miR-499) and cardiac tissue (miR-24) in addition to those involved in inflammation (miR-21, miR-146a), angiogenesis (miR-210, miR-222), and hypoxia/ischemia adaptation (miR-21, miR-146a, miR-210) were measured before, directly after, and 24 hours after exercise. The fold changes in c-miRNAs were compared using both the Friedman test followed by Dunn's multiple comparison test and a linear mixed model adjusted for age and peak V_{O2}.
Results: Directly following exercise, c-miR-1 increased significantly (3.79 ± 0.60-fold change, $p < 0.02$) at peak pace and followed a highly significant dose-dependent trajectory ($p < 0.01$), increasing with exercise intensity. On the other hand, with all exercise intensities, c-miR-133a increased immediately after exercise but did not display a dose-dependent response at this time point. c-miR-133a, however, decreased significantly (0.93 ± 0.18-fold change, $p < 0.03$) 24 hours following exercise at peak pace but not at other exercise intensities. Although c-miR-222, c-miR-24, and c-miR-146 levels increased directly following exercise, their responses otherwise did not vary with exercise intensity. Finally, c-miR-206, c-miR-499, c-miR-210, and c-miR-21 remained stable among time points at all intensities.
Conclusions: c-miR-1 and c-miR-133a both display dose-dependent responses to varying intensities of acute aerobic exercise – the former increasing with speed directly after exercise and the latter decreasing with speed 24 hours following exercise. The specificity of these responses indicates that unique mechanisms dependent upon exercise intensity regulate the secretion and clearance of these c-miRNAs. As such, these findings support the potential development of these miRNAs as biomarkers of cardiovascular fitness.

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Natural Aromatic Amino Acid Substitution Stabilizes Insulin Hexamers

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Insulin is a peptide hormone secreted by pancreatic β-cells in response to increased interstitial glucose levels. By binding to its molecular target, the insulin receptor (IR), insulin initiates the uptake of glucose by peripheral tissues. The absolute or relative lack of insulin is responsible for the medical conditions designated Type I Diabetes Mellitus (T1DM) and Type II Diabetes Mellitus (T2DM), respectively. Insulin replacement therapy is the primary treatment for T1DM and supplemental insulin administration is a major component in T2DM treatment. The insulin hormone has developed a number of structural adaptations that protect it from unfolding or misfolding during synthesis and storage. Among these is the ability of the molecule to self-associate, forming dimers and zinc-coordinated hexamers. The C-terminal B-chain contributes to the stability of the insulin monomer by packing against the hydrophobic core of the protein, and it comprises a majority of the monomer-monomer interface of the insulin dimer. Residue tyrosine B26, part of the "aromatic triplet" of residues, is highly conserved across insulin-expressing taxa because of its critical role in stabilizing the C-terminal B-chain of insulin. In this study, we explore the substitution of tryptophan, a large aromatic residue, for the native tyrosine at the B26 position and its consequences on the structural stability and biological activity of insulin. Metal-ion absorption spectroscopy of the phenol-stabilized (R_6) insulin hexamer revealed a remarkable increase in hexamer stability: half-life of Trp^{B26} hexamers was found to be over 100 fold greater than that of native insulin. Although size-exclusion chromatography confirmed the increased hexamerization capability of Trp^{B26} insulin, the analog was shown to have a native-like tendency to dimerize. Circular dichroism studies indicated similar thermodynamic stability of Trp^{B26} monomers to those of native insulin. Trp^{B26} insulin retains native affinity for IR in vitro and native-like biological activity as determined by intravenous injection in diabetic rats. However, the analog displays a decreased rate of blood-glucose clearance compared to native insulin when injected subcutaneously in diabetic rats. For this reason, Trp^{B26} insulin may be a candidate for future therapeutic long-acting (basal) insulin analogs.

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Gene Networks Implicate Microglial-mediated Inflammation in Frontotemporal Dementia (FTD)

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Growing evidence supports a direct role for neuroinflammation in neurodegeneration and yet few studies have analyzed this process in a systematic manner. We used RNA sequencing to define a set of gene expression networks robustly associated with frontotemporal dementia (FTD) in mouse models harboring mutations in the microtubule associated protein tau (MAPT). We combined these data with data collected across different stages of disease progression in other mouse models and in human FTD post-mortem specimens at the RNA and protein levels. We next applied drug-gene network interaction databases to identify potential network activators and inhibitors, identifying several drugs known to ameliorate disease in AD or FTD mouse models, as well as drugs in clinical trials for AD and ALS. These results largely derived from cancer cell lines are promising, and yet are limited because their neural relevance is uncertain. We are addressing this challenge by testing the drug-network predictions in microglial cultures derived from the central nervous system, where we can also understand how perturbed gene networks relate to microglial functions such as phagocytosis. The driving hypothesis is that a better understanding of these drug-gene network relationships in microglia will enhance our ability to translate these findings to usable therapeutics for nervous system disorders. This work is sponsored by Takeda pharmaceuticals.

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Regulation of Cardiomyogenesis by TGF-β signaling

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Heart disease is the leading cause of death in the United States. Over half of all cases of heart disease are caused by coronary artery disease, which can lead to myocardial infarction (MI) and heart failure. Current standards of care including pharmacological inhibition of β-adrenergic receptors ("β-blockers") demonstrate improved ventricular function and long-term survival in patients; however, these therapies are unable to stop or reverse the progression of heart failure due to the inability to regenerate cardiomyocytes (CMs) following coronary events. Cell therapies including delivery of induced pluripotent stem cells (iPSCs) and direct reprogramming of fibroblasts to CMs offer the potential to regenerate the heart following MI. However, translation to humans faces numerous challenges including increasing the efficiency of generating CMs, generating a homogeneous, mature population of CMs, and overcoming safety concerns for delivery to human hearts. Our lab recently demonstrated efficient generation of CMs from fibroblasts (direct reprogramming) by overexpressing GATA4, Hand2, Mef2c, Tbx5, miR-1, and miR-133 (GHMT2m) alongside TGF-β receptor 1 (TGFR1) inhibition. Importantly, the mechanisms by which TGFR1 inhibition increases CM regeneration efficiency remain poorly understood. My preliminary studies indicate that GATA4, Hand2, and Tbx5 (GHT) physically interact with the H3K27me3-specific JMJD3. Furthermore, TGF-β signaling impairs these interactions. Recent publications demonstrate that JMJD3 also physically interacts with Smad2/3. Therefore, physical interactions between GHT and JMJD3 may be disrupted by TGF-β signaling effectors Smad2/3, impairing CM regeneration. Smad2/3 compete with cardiac transcription factors to bind and/or recruit JMJD3 to cardiac gene loci, impairing with CM regeneration. **Methods:** Using biochemical assays including co-immunoprecipitation and chromatin immunoprecipitation, I aim to establish the mechanism by which TGF-β signaling impairs CM regeneration. Additionally, the functional roles of GHT interactions with JMJD3 will be tested in multiple models of CM regeneration. **Conclusions:** Despite the success of medical intervention in slowing the progression of heart failure and fibrosis following MI, the 5-year mortality rate in patients remains near 50%. Furthermore, regenerative capacity in the adult heart is low. Unraveling the mechanisms governing CM regeneration could therefore lead to novel therapeutic strategies to renew the CM population in diseased hearts.

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IL-15 Sustains IL-7R-independent ILC2 and ILC3 Development

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Innate lymphoid cells (ILCs) are mucosal sentinels that produce T helper cell cytokines directly in response to cytokine signals in the microenvironment without a need for antigen-specific receptor signaling or preactivation. The signals that maintain tissue-resident innate lymphoid cells in different microenvironments are incompletely understood. Currently, ILC2 and ILC3 are thought to require IL-7, while ILC1 and NK cells require IL-15. Here, we test the extent to which ILCs require IL-7 receptor (IL-7R) across mucosal tissues. IL-7R is not strictly required for the development of any ILC subset, as residual ILC2 and ILC3 cells persist in the small intestine lamina propria (siLP) of adult and neonatal *Il7ra*$^{-/-}$ mice. All ILC subsets are functionally competent *in vitro*, and are sufficient to provide enhanced protection to infection with *C. rodentium in vivo*. Compared to other common gamma chain cytokines, *Il15* is expressed at higher levels in mucosal tissues. IL-15 equally sustained wildtype and *Il7ra*$^{-/-}$ ILC survival *in vitro* and compensated for IL-7R deficiency, as residual ILCs are depleted in mice lacking both molecules. Collectively, these data demonstrate that siLP ILCs are not completely IL-7R dependent, but can partially persist through IL-15 signaling.

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Benefits of Combinatorial Therapies for Improving Functional Recovery in a Rat Model of Facial Nerve Injury

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Injury to the facial nerve due to trauma or surgical intervention for head and neck pathologies is common; however, few effective treatments eliciting full functional recovery following facial nerve injuries exist. Previous studies in our laboratory have found that single-agent therapies, while effective in improving functional recovery, are insufficient in promoting complete functional recovery after nerve injury. Consequently, combination therapies are an area of emphasis in our research. We have found that treating the repaired injured nerve with polyethylene glycol (PEG), a sealant that acts to fuse damaged cell membranes, and methylene blue (MB), an antioxidant, improves functional recovery in a rat model of facial nerve transection and suture. Given the ease in application of these therapies, either singularly or in combination, we anticipate that these and future studies will aid translation of such methods for application in human facial nerve injury patients. Supported by a Roudebush VA IIMR Young Investigator Award (CLW) and IUCRG grant from IUSM (KJJ).

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Hidden Markov Model Analysis of Transposon Mutagenesis in Fission Yeast

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Recent advances have highlighted non-coding regions of the genome (e.g. miRNAs) that act as regulatory elements in the cell. A major issue is to identify functional sequences within non-coding regions and their purpose. We aim to identify non-coding regions important in growth and ageing by applying transposon mutagenesis in combination with an annotation free analysis in S. pombe. The Hermes transposon artificially introduces a transposable element into the yeast genome in a stochastic manner. Cells are grown during and beyond transposon mutagenesis, causing those with insertions in essential regions to display hindered growth. This results in selection of fit cells, observed as a lack of insertions in functional regions. Thereafter a chronological life span assay is used to investigate the impact of transposon mutagenesis on aging. Following nutrient restriction, cells approach stationary phase and decline in population. Transposon insertions differentially affect cell aging by elongating or shortening the life span, observed as a gradual or rapid decrease of transposons over time respectively. A hidden markov model was used to mathematically analyse the data, taking hermes insertion biases into account and define regions as essential, intermediate and non-essential for fitness, based on the observed insertions. The analysis reveals previously unannotated regions that appear to be crucial for the fitness of the cell, highlighting some specific non-coding regions, signaling their importance in regulation.

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Selective Stimulation of Facial Muscles Following Chronic Intraneural Electrode Array Implantation and Facial Nerve Injury in the Feline Model

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Our group has previously shown that activation of specific facial nerve (FN) fiber populations and selective contraction of facial musculature can be achieved through acute intra-neural multi-channel microelectrode array (MEA) implantation in the feline model. Selective stimulation of facial muscles will be maintained in the setting of (1) chronic and (2) acute MEA implantation following FN injury recovery. **Methods:** This study included seven cats. In three cats with normal facial function, chronic intraneural implantation was performed and tested biweekly for six-months. Electrical current pulses were delivered to each channel individually, and elicited electromyographic (EMG) voltage outputs were recorded for each of several facial muscles. For FN injury experiments, two cats received a standardized hemostat-crush injury, and two cats received a transection-reapproximation injury to the FN main trunk. These four underwent acute implantation of a 4-channel penetrating MEA

four-months post-injury. **Results:** Stimulation through individual channels selectively activated restricted nerve populations, resulting in versatile contraction of individual muscles in cats with chronic array implantation and following nerve injury. Increasing stimulation current levels resulted in increasing EMG voltage responses in all cases. Nerve histology showed minor neuronal tissue reaction to the implant. **Conclusion:** We have established in the animal model the ability of a chronically implanted MEA to selectively stimulate restricted FN fiber populations and elicit contractions in specific facial muscles. Likewise, following FN injury, selective stimulation of restricted FN fiber populations and subsequent contraction of discrete facial muscles can be achieved following acute MEA implantation.

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Enhanced Training-induced Recovery in Subacute Occipital Stroke Patients

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Occipital stroke that damages primary visual cortex causes a homonymous loss of conscious vision known as cortical blindness (CB). Patients with CB are impaired with respect to most activities of daily living including reading, navigating, recognizing people and objects, and driving. Currently, there are no effective, clinically implemented vision restitution therapies for CB and the vision loss is assumed to be permanent. However, over the past decade, our lab has shown that even in chronic CB (>6 months post-stroke), vision can be recovered using gaze-contingent visual training. Although a hugely positive outcome, the required training is lengthy and the recovered vision is not completely normal. Evidence from sensorimotor stroke suggests that earlier rehabilitation leads to greater recovery, likely because the post-injury environment favors neuroplasticity. Here, we asked if visual training initiated sooner than 6 months after stroke leads to better improvement in CB. Ten subacute patients 1-3 months post-stroke were recruited and trained to discriminate global motion direction of random dot stimuli in their blind field. Initially, blind field performance was at chance (mean normalized direction range [NDR] threshold=0 degrees). To date, four subacute subjects enrolled have completed their assigned daily home training over a period of 5 ± 1 months. Three subacutes underwent enrollment and baseline testing but were unable to initiate training correctly due to computer difficulties, and the remaining three are still completing training. The four subacute CBs who have completed training attained normal motion integration performance (NDR=34 ± 13 degrees) at their trained blind field locations. They also reached this normal NDR faster than chronic CBs (subacutes in 10 ± 15 sessions; chronics in 58 ± 45 sessions) Additionally, unlike chronic CBs who never transfer learning more than 1 degree deeper into the blind field, subacute CBs exhibited transfer of recovery to untrained locations 7 ± 3 degrees deeper into the blind field. Thus, training initiated in subacute CB patients generates faster, more spatially generalized visual improvements than identical training initiated in chronic CB, and may represent a more efficient rehabilitation strategy for vision loss after occipital stroke.

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A Histological Comparison of Ear Skin Regeneration in *Acomys* and *Mus*

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Regeneration has been studied almost exclusively in lower invertebrates as most mammals are only able to regenerate fetal tissue. The African spiny mouse (*Acomys*) represents the first time advanced regeneration has been observed in an adult mammal. *Acomys* has evolved a defense mechanism which involves fragile skin that tears easily when caught by a predator, allowing the mouse to escape. Subsequently, the mouse regenerates extensive parts of its body. The regenerative capabilities of *Acomys* are being studied by comparing it to a normal mouse (*Mus*). In order to compare the progression of ear regeneration in *Acomys* and *Mus*, ears of both species were wounded using a four-millimeter punch to remove the epidermal and dermal tissue layers, revealing the underlying cartilage. The healing ears were harvested at 2, 4, 7, 14, 21, and 30 day time points. The ears were subsequently embedded in wax, mounted on slides, and trichrome stained to differentiate between erythrocytes, muscle and collagen. Microscopic analysis revealed that although the cartilaginous layer eventually degenerated in both species, extensive degeneration was present much earlier in *Mus*. Furthermore, *Acomys* was able to regenerate its cartilage and hair follicles, whereas *Mus* was only able to regenerate a disorganized, nonfunctioning mass of collagen. Significant scarring was evident in Mus, while no scarring was observed in *Acomys.* The results of further study of *Acomys* could prove integral in gaining a comprehensive understanding of the regenerative process. Findings could ultimately improve the entire healthcare field by allowing for the regeneration of human tissue.

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Mapping the Polar Angle Representation of Saccades in Human Superior Colliculus

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Purpose: The superior colliculus (SC) is a layered midbrain structure involved in directing eye movements and coordinating visual attention. Electrical stimulation and neuronal recordings in the intermediate layers of monkey SC have revealed a retinotopically organized saccadic eye-movement map. However, the polar angle representation of saccades in ***human*** SC has not been well studied. We used high-resolution fMRI to map the representation of eye movements in human SC. **Methods:** We implemented a phase-encoding approach similar to previous human studies of saccadic mapping. However, these studies operated with a very low duty cycle (1 saccade every 5 s) and reverse saccades made immediately after forward saccades. We designed a novel paradigm in which subjects performed multiple forward saccades, then returned to the opposite visual field using guided smooth pursuit. Subjects made series of saccades either to left or right (activating primarily the contralateral SC) while we cyclically varied the vertical component of the cue to correspond to saccades in the lower, horizontal, and upper visual field. Attention was engaged via a target discrimination task at the end-point of every

saccade and along smooth pursuit. Eight quasi-axial slices covering SC were imaged on a Siemens 3T Trio (2.4 sec/volume, 1.2-mm voxels). Each run consisted of 9, 28.8-s-duration cyclic repetitions of the 3 saccade directions; imaging sessions included 14—16 runs which were subsequently averaged. Sinusoids were fit to the data, yielding phase data that related the fMRI response to saccade angle. Phase encoding with moving-dot stimuli measured the polar-angle representation of the visual field in SC. **Results**: The expected lateral-to-medial phase progression was observed in each of 5 participants. Also, we found the saccadic maps lie deeper in the SC (intermediate layers) and are in alignment with the more superficial visual-field retinotopy. **Conclusion**: Our techniques in psychophysics and imaging allow us to relate findings in non-human primates to human SC, strengthening our understanding of subcortical vision.

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Transcriptional Responses to Lipopolysaccharide, Poly I:C and House Dust Mite Extract of Tissue Resident Memory Human Lung CD4 and CD8 Tissue Resident Memory T Cells in Human Lungs

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Tissue resident memory (TRM) CD4 and CD8 lung T cells have recently been identified as T cells that do not circulate in the periphery and likely play a crucial role in the protection against microbial infection. Asthma is a disease where exacerbations are frequently triggered by viral or bacterial infection and allergen exposure. As T cells are a major contributor to asthmatic airway and lung inflammation, TRM T cells are likely important in this disease. CD4 TRM populations were identified by flow cytometry as CD3+ CD4+ CCR7- CD45RO+ CD11a+ CD69+, and CD8 TRM populations were identified as CD3+ CD8+ CCR7- CD45RO+ CD103+ CD69+. The frequency of these populations was determined in human donor lungs that were not used for transplant from 10 individuals with asthma and 10 individuals without asthma. In additional experiments, lung leukocytes were treated with lipopolysaccharide (LPS), poly I:C and house dust mite (HDM) extract for 20 hours to study responses to bacterial, viral and allergen exposure, respectively. After treatment, CD4 and CD8 TRMs were sorted using fluorescence activated cell sorting. RNA sequencing was performed on each cell type after exposure to each treatment and a media control. In lungs from non-asthmatic individuals, the proportion of lung CD4 TRMs was highly variable, ranging from 0.09% to 1.2% of CD45+ cells; the proportion of CD8 TRMs ranged from 0.03% to 0.3% in this group. In samples from asthmatic individuals, the proportion of TRMs was also variable, ranging from 0.009% to 1.9% for CD4 TRMs and 0.03% to 0.7% for CD8 TRMs. In the leukocyte culture experiments, LPS exposure resulted in gene expression changes in 8.6% of all genes in CD4 TRMs, whereas HDM or PIC treatment resulted in less than 1% of genes changing expression levels at least two-fold compared to the untreated cells. In contrast, in CD8 TRMs 8.7%, 5.7% 10.8 of genes had more than two-fold change compared with untreated cells with LPS, HDM and poly I:C treatment, respectively. Ingenuity Pathway Analysis performed using lists of genes with the largest difference in expression for each treatment identified unique networks enriched for cell trafficking, chemokine, cytokine and costimulatory molecules. In conclusion, TRMs comprise a small fraction of total lung leukocytes, with specific transcriptional responses within 20 hours of stimulation with TLR agonists or allergens that mimic bacterial, viral or allergen exposure.

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Altered Sphingolipid Metabolism and Mitochondrial Dysfunction in Charcot Marie Tooth 2F

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Charcot-Marie-Tooth (CMT) disease is the most commonly inherited neurological disorder, resulting in motor and sensory dysfunction that typically commences at middle age and progressively worsens throughout life. Nevertheless, the exact molecular mechanisms underlying CMT are unclear, limiting progress in developing potential therapeutics. As sphingolipids serve as bioactive signaling molecules in a plethora of pathways and have been implicated in neurodegenerative diseases including Alzheimer's, Parkinson's, and Amyotrophic Lateral Sclerosis, we sought to determine if changes in sphingolipid metabolism may mediate CMT phenotypes. One variant of CMT, CMT2F, is characterized by the presence of mutations in heat shock protein 27 (Hsp27), a member of the class of small heat shock proteins that serves many cellular functions. Using liquid chromatography/mass spectrometry, we have determined that mutant Hsp27 mice display decreased ceramide in sciatic nerve but not brain tissue compared to wild-type Hsp27 mice, suggesting CMT2F pathology may occur due to decreased ceramide levels. Mutant mice show loss of mitochondrial populations of Ceramide Synthase 1, the predominant ceramide synthase enzyme in the nervous system. Mitochondria of cells transfected with these mutants display increased area and interconnectivity, paralleling changes induced by blocking ceramide generation, suggesting decreased production of ceramide alters mitochondrial dynamics. Functionally, mitochondrial basal and maximal respiration and spare respiratory capacity are decreased in mutants, also matching changes induced by blocking ceramide generation. Furthermore, autophagy is increased in mutant cell lines, suggesting a potential mitophagy phenotype. Taken together, these results suggest that CMT2F mutations in Hsp27 alter sphingolipid metabolism by dysregulating ceramide levels, producing mitochondrial pathology that ultimately leads to neuronal degeneration in CMT2F. By understanding the effects of sphingolipids on the development of CMT2F, we hope to be able to identify targets that can be used in the development of novel treatments to prevent or reduce the severity of CMT2F or other neuropathies with similar mechanisms.

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An Aberrant Immunosuppressive Signature within Bone Marrow May Identify Patients Prone to Recurrence of Metastatic Prostate Cancer after Prostatectomy

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Prostate cancer is the second leading cause of cancer-related deaths in US men, with metastatic disease representing a lethal event. Despite treatment with curative intent with surgery or radiation, nearly one-third of men develop metastatic disease. Early dissemination of tumor cells to the bone metastatic niche has been hypothesized as the underlying cause of recurrent cancer. Bone metastases comprise the majority of distant disease, and cause significant morbidity in addition to driving mortality rates. The factors that promote early dissemination, mediate survival in the bone marrow microenvironment, and induce late reactivation, are not clearly elucidated. Immune evasion has been hypothesized as a critical mechanism by which tumor cells survive and proliferate in metastatic lesions. This phenomenon is even more critical in the complex immune microenvironment found in bone marrow. **Methods:** To evaluate mechanisms of immune evasion that promote the development of metastatic prostate cancer, blood and bone marrow aspirates were collected from 13 patients undergoing prostatectomy for presumed localized disease. Using multicolor flow cytometry, the immune compartment in matched blood and bone marrow samples were examined for the percentage of CD4 and CD8 T cells, B cells, CD14 monocytes, and Foxp3-positive regulatory T cells that may regulate anti-tumor immune responses. Matched samples were processed for analysis of circulating and disseminated tumor cells in blood and bone marrow, respectively. **Results:** In our initial cohort, we identified a subset of patient samples with low T cell to B cell ratios ranging from as low as 1:1 to as high as 9:1. A high CD4 to CD8 T cell ratio was identified in 38% of samples tested that corresponded with a higher occurrence of regulatory T cells ranging up to 13% of CD4+ events. This signature was not present in the matched peripheral blood samples. A subset of samples also showed evidence of disseminated tumor cells corresponding with immunosuppressive signatures. **Discussion:** Overall, these results support the hypothesis that an immunosuppressive signature within the bone marrow may promote survival of disseminated prostate cancer cells by protecting them from eradication by effector T cells, thus priming a population of patients to be predisposed to developing recurrent prostate cancer after prostatectomy. Furthermore, these biomarker analyses can be correlated with an immune signature within the microenvironment to identify factors that promote dissemination and metastatic recurrence. Importantly, this permissive microenvironment may already be present at the time of prostatectomy, potentially allowing clinicians to identify those patients that would benefit from aggressive upfront adjuvant systemic therapy.

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Missense Mutations in *SMCHD1* are Associated with Isolated Arhinia, Bosma Arhinia Microphthalmia Syndrome, and Facioscapulohumeral Muscular Dystrophy Type 2

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Arhinia (absent nose) is a rare congenital malformation that occurs in isolation or with ocular and neuro-reproductive defects, a triad called Bosma Arhinia Microphthalmia syndrome. The genetic cause is unknown. **Methods:** We assembled a cohort of 40 patients with arhinia and used next generation sequencing, rare mutation burden testing, and functional modeling to identify the etiology of this disorder. **Results:** *The phenotypic spectrum:* Arhinia was typically accompanied by a high-arched/cleft palate, hypoplastic sinuses and maxilla, nasolacrimal duct and choanal atresia, and ocular defects (an- or micro-ophthalmia, uveal coloboma, cataract). The reproductive axis was assessed in 31 subjects and 97% demonstrated hypogonadatropic hypogonadism. Olfactory structures were absent in all subjects with brain imaging. *Gene discovery:* We identified rare, heterozygous missense *SMCHD1* variants in 86% of cases. All variants were located in the SMCHD1 ATPase domain which we determined to be under strong evolutionary constraint using regional constraint models. Gene-based burden testing of rare variants confirmed that *SMCHD1* was the only gene to achieve genome-wide significance ($p=2.9x10^{-17}$). *Pleiotropy and oligogenic etiology:* This discovery was unexpected as loss-of-function mutations in *SMCHD1*, in combination with a permissive 4q35 haplotype and truncated D4Z4 repeat, cause a rare form of muscular dystrophy (FSHD2). SMCHD1 is an epigenetic repressor that maintains X-inactivation and silences autosomal gene clusters. In FSHD2, loss of SMCHD1 function leads to D4Z4 hypomethylation and abnormal expression of the muscle toxin, DUX4. Variants associated with FSHD2 span the entire gene and include missense and truncating mutations; variants associated with arhinia were exclusively missense and within the ATPase domain. However, several FSHD2-specific mutations also localized to the ATPase domain, and at least two FSHD2 variants were detected in our arhinia cohort. The arhinia mutations had the same direction of effect as reported for FSHD2 mutations: 74% of arhinia cases with an *SMCHD1* variant had D4Z4 hypomethylation characteristic of FSHD2 while family members without an *SMCHD1* variant did not. Further analyses identified two arhinia patients and a father who met all genetic requirements for FSHD2; one patient has asymmetric muscle atrophy and the father is being treated for muscular dystrophy. *Functional studies:* Transient and permanent (CRISPR/Cas9) ablation of the SMCHD1 locus in zebrafish caused abnormal facial cartilage, small eyes, and blunted GnRH-immunopositive terminal nerve projections. Each phenotype was rescuable with wild-type but not with mutant *SMCHD1* mRNA, demonstrating assay specificity. RNAseq and gene-set analyses in human cell lines revealed down-regulation of genes statistically enriched for one phenotype: “depressed nasal tip”. **Conclusions:** Rare variants in an evolutionarily constrained

region of *SMCHD1* are associated with arhinia. Mutations in *SMCHD1* also cause an oligogenic form of muscular dystrophy, demonstrating a strikingly diverse phenotypic spectrum from identical alleles and implicating disruption of critical interactions with other loci.

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Ventriculo-Amniotic Shunt for Fetal Aqueductal Stenosis (VASFAS)

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Neonatal hydrocephalus due to aqueductal stenosis is a debilitating disease characterized by an excessive accumulation of fluid within the brain. Current treatment consists of a postnatal shunt, but this does not prevent poor neurological outcomes. VASFAS, a prenatal shunt which can be placed during pregnancy to prevent the buildup of pressure from fluid in the brain, may drastically decrease the morbidity, mortality and cost of overall care of these children. Fetal aqueductal stenosis affects an estimated 25-60,000 infants annually on a global basis. The application of the device can be widened to treat conditions such as lower urinary tract obstruction and pressure hydrothorax, both of which are of similar incidence to aqueductal stenosis. A provisional patent application has been filed with the University of Pittsburgh (Application No. 62/241,281) on October 14, 2015. Our collaborators are Center for Medical Innovation, Clinical and Translational Science Institute, McGowan Institute of Regenerative Medicine, Pittsburgh. In the future, we plan to refine the prototype and establish its safety in animals.

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Omics Profiling of Tracheal Aspirates Reveal Novel Biomarkers of Pulmonary Hypertension of the Newborn

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In the past decade, neonatal care has drastically improved resulting in survival of extremely premature babies. Consequently, the incidence of bronchopulmonary dysplasia (BPD) and associated comorbidities has significantly increased. Persistent pulmonary hypertension of the newborn (PPHN) in the setting of BPD has high mortality and morbidity due to a lack of early diagnostic markers. Cardiac catheterization is a highly invasive procedure and is the gold standard for diagnosing PPHN. Echocardiogram and serum Brain Natriuretic Protein levels are less accurate. **Objective:** The goal of this study was to evaluate miRNA and proteomic profiles in tracheal aspirates (TAs) of infants to find potential biomarkers that can be used to identify the onset of PPHN. **Methods**: We collected TAs from infants receiving mechanical ventilation at the Penn State Children's hospital NICU. Patients (n=65) had a confirmed clinical diagnosis of BPD (n=35), BPD+PPHN (n=10), or no evidence or history of lung disease (controls, n=20). The expression of >1000 human miRNAs was quantified with the miScript miRNA Human miRNome array (Qiagen), and the proteomic profile was analyzed by mass spectrometry. **Results:** Analysis of miRNA and proteomic profiles identified specific signatures for each disease group. There were 16 miRNAs with at least 4-fold difference in PPHN patients vs. controls. The most significantly differentially expressed miRNAs among groups were miR-490-3p, miR-450b-3p, and miR-507. Furthermore, mass spectrometry analysis identified 14 differentially expressed proteins in the PPHN group vs. BPD and controls. Of these, catalase, IGLL, S100A8, BPIFB1, and clusterin showed the greatest differences. Ingenuity Pathway Analysis (IPA) predicted associations of these proteins with pro-inflammatory pathways involving iNOS, IFNgamma, JAK, and STAT protein expression. The IPA miRNA analysis showed interactions with TP-53 and CDKN1A, which are cell cycle regulators, as well as involvement of NF-kB in pro-inflammatory pathways. To our knowledge, this is the first study to identify specific miRNA and proteomic profiles in tracheal aspirates from PPHN patients. IPA analysis suggested the involvement of multiple pathways that control inflammatory and morphometric characteristics of the disease. These may reveal specific candidate genes involved in the disease pathogenesis and represent potential novel non-invasive biomarkers for early disease diagnosis. **Conclusion:** To our knowledge, this is the first study to identify specific miRNA and proteomic profiles in tracheal aspirates from PPHN patients. IPA analysis suggested the involvement of multiple pathways that control inflammatory and morphometric characteristics of the disease. These may reveal specific candidate genes involved in the disease pathogenesis and represent potential novel non-invasive biomarkers for early disease diagnosis.

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A Patient-Centered Emergency Department Management Strategy for Sickle-Cell Disease Super-Utilizers with Vaso-Occlusive Pain Crisis

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A subpopulation of sickle-cell disease patients, termed super-utilizers, present frequently to emergency departments (EDs) for vaso-occlusive events and may consume disproportionate resources without broader health benefit. To address the health care needs of this vulnerable patient population, we piloted a multidisciplinary intervention seeking to create and use individualized patient care plans that to alter utilization through coordinated care. Our goals were to assess feasibility primarily, and to assess resource use secondarily. **Methods*:*** We evaluated the effects of a single-site interventional study targeted at a population of adult sickle-cell disease super-utilizers using a pre- and post-implementation design. The pre-intervention period was 06/01/13 to 12/31/13 (7 months) and the post-intervention period was 01/01/14 to 02/28/15 (14 months). Our approach included: patient-specific best practice advisories (BPA); an ED management protocol (figure 1); formation of a "medical home" for these patients. **Results*:*** For 10 subjects targeted initially we developed and implemented coordinated care plans; after deployment, we observed a tendency toward reduction in ED and inpatient utilization across all measured indices. Between the annualized pre- and post-implementation periods: ED visits decreased by 16.5 visits/pt-yr (95% CI, -1.32 to 34.2); ED length of state (LOS) decreased by 115.3 hours/pt-yr (95% CI, -82.9 to 313.5); in-patient admissions decreased by 4.20 admissions/pt-yr (95% CI, -1.73 to 10.1); in-patient LOS decreased by 35.8 hours/pt-yr (95% CI, -74.9 to 146.7); and visits where the patient left before treatment was reduced by an annualized total of 13.7 visits. We observed no patient mortality in our 10 subjects and no patient required admission to the ICU care 72 hours following discharge. **Conclusion*:*** This effort suggests that a targeted approach is both feasible and potentially effective, laying a foundation for broader study.

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GATA6 Regulates Immune-related Genes in Endometriosis

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Endometriosis is an estrogen-dependent, gynecological disease that affects 5–10% of reproductive-age women. The main symptoms are severely painful menses, chronic pelvic pain, painful intercourse, and more commonly, infertility. The mechanism through which normally-located eutopic endometrial cells (NoEM) are associated with endometriotic cells (OSIS) remains unknown. Recent genome-wide methylation analyses from our lab have pinpointed a unique epigenetic fingerprint in endometriosis, suggesting DNA methylation is an integral component of the disease. We observed significant differences between NoEM and OSIS in DNA methylation of the GATA family of transcription factors, suggesting a novel role for the GATA family as key regulators of uterine physiology. When GATA6 is expressed in NoEM, there is a lack of developmental plasticity, a block in hormone sensitivity, and an induction of endometriosis markers. We developed two groups consisting of primary cells for ChIP-Seq: 1) NoEM overexpressing GATA6 via adenoviral vector and 2) OSIS control. For RNA-Seq, our two groups consisted of 1) NoEM control and 2) NoEM overexpressing GATA6 via adenoviral vector. By conducting analyses on our ChIP-Seq and RNA-Seq data, we were able to determine that GATA6 modulates many immune genes and pathways. We observed GATA6 was highly enriched at the promoters of immunomodulatory genes via ChIP-Seq. Concurrently, our RNA-Seq results indicated differential expression of many immune-related genes. Examples of these genes include chemokines (e.g., RANTES and CCL2) and interleukins (e.g., IL6). Our results coincide with several observations of inflammation and pain in patients with endometriosis and apoptotic resistance in OSIS. Overall, endometriotic cells possess a unique immunoregulatory, anti-apoptotic, and steroidogenic phenotype that directly contributes to the survival and persistence of diseased tissue. Our data suggest that differential DNA methylation directing the expression of GATA6 contribute to this diseased phenotype. This study uncovers a potential epigenetic and immunologic basis for GATA6 action in endometriosis, proposing a usefulness for the development of targeted and effective therapies for this disease.

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Brain-Adipocyte Axis Activation by Gene Transfer Treats Melanocortin-4-Receptor Obesity

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Obesity is a major pandemic affecting over one-third of the U.S. population, with not only economic but also health costs in the form of higher risk for developing other chronic illnesses, such as cardiovascular disease and cancer. Mutations in the melanocortin-4-receptor (*MC4R*) comprise the most common monogenetic form of severe early-onset obesity, and conventional weight-loss treatments for these patients are either ineffective long-term or contraindicated. Besides genetics, environmental factors and lifestyle have profound roles on metabolism. Recently, environmental enrichment (EE), a complex housing condition that augments mental health, has revealed a novel antiobese phenotype characterized by reduced adiposity and resistance to diet-induced obesity. The mechanism is partly due to hypothalamic-sympathoneural-adipocyte axis activation, whereby upregulated brain-derived neurotrophic factor (*Bdnf)* in the arcuate and ventromedial hypothalamus leads to increased sympathetic tone, preferentially onto adipose tissue. Our previous work demonstrated that adeno-associated viral (AAV)-mediated delivery of *Bdnf* to hypothalamus mimicked the effects of EE. Specifically, hypothalamic injections of AAV-*Bdnf* attenuated weight gain in genetic mouse models of obesity, in which genes mutated lie upstream of MC4R signaling. Furthermore, BDNF lies downstream of MC4R, so *Bdnf* gene transfer may also treat obesity in *Mc4r* mutants. We therefore hypothesize that Bdnf gene transfer to the arcuate/ventromedial hypothalamus of Mc4r-deficient mice will mimic the antiobese effects of EE without adverse side effects. To address this, 3-month-old female *Mc4r*$^{+/-}$ mice were injected bilaterally with either AAV-*Bdnf* or AAV-YFP (yellow fluorescent protein) as control. Next, body weight was monitored weekly to observe short-term metabolic efficacy. Glucose and insulin tolerance and echoMRI were performed later to assess long-term metabolic improvements. Blood pressure was monitored at 28 weeks post-injection to determine long-term safety. Altogether, the results demonstrated decreased body weight, improved metabolic function, and reduced adiposity in the *Bdnf*-treated mice relative to controls. No significant difference in systolic or diastolic blood pressure was observed between the two groups. In conclusion, these studies demonstrate the metabolic efficacy by which *Bdnf* gene transfer attenuates obesity in *Mc4r*$^{+/-}$ mice. Preliminary safety findings suggest no adverse cardiovascular function due to this therapy. Future directions include histological imaging of organs, serum analysis, performing similar experiments on *Mc4r*$^{+/-}$ male mice, and additional safety studies, including investigating nervous system side effects. The safety and efficacy data from these studies will hopefully provide sufficient preclinical evidence that hypothalamic *Bdnf* gene transfer is a viable therapeutic option in the future for *MC4R*-deficient obese patients.

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Chloroquine Altered Polysaccharide Nanoparticles for Simultaneous Sensitization and Oxaliplatin Delivery to Pancreatic Cancer

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Significance: Pancreatic cancer (PC) has the lowest survival after diagnosis of all common cancer types. Despite continued efforts, PC has overtaken breast cancer as the third leading cause of cancer-related mortalities and will overtake colon cancer for second by 2030. With current therapies, PC survival is still less than a year, which is a consequence of ineffective accumulation of chemotherapeutics in tumors and the highly chemoresistant nature of PC. Formulating therapeutics with a drug delivery system (DDS) can enhance tumor accumulation of chemotherapeutics, but because of the chemoresistant nature, delivering a drug to sites of PC may not be adequate. Many preclinical and phase I/II trials have shown that combination therapies with chloroquine (CQ) sensitize cancer cells and prevent drug resistance to chemotherapeutics including oxaliplatin (OX). We can improve the combination of CQ and OX by creating a CQ-modified nanoparticle DDS, termed Chloroquine-Altered PolySaccharide (CAPS), that can encapsulate and deliver OX (Chemo-CAPS). The significance of this research is that Chemo-CAPS will enhance PC tumor accumulation of both CQ and OX thereby sensitizing and improving OX anticancer activity. Proper nanoparticle formulation of Chemo-CAPS will co-deliver CQ and OX leading to synergistic enhancement in therapeutic efficacy. **Experimental Design:** CAPS was synthesized by conjugating CQ to hydroxyethyl starch and characterized via GPC, NMR, elemental analysis, DLS, and AFM. Live cell imaging and LC3 levels assessed autophagy inhibition and intracellular trafficking. Migration and invasion studies examined the effects on cellular behaviors in AsPC-1, Mia Paca-1, and Mia Paca-2 PC cell lines. Colo-357 orthotopic mouse models assessed the *in vivo* effects on tumor growth and metastasis of CAPS. Using the solvent displacement method, OX-loaded Chemo-CAPS nanoparticles were prepared and characterized using the previous methods to assess the physical and biological properties of Chemo-CAPS. **Results**: DLS and AFM confirmed that CAPS was able to self-assemble into nanoparticles with a mean diameter of 15 nm with a narrow size distribution. Live cell imaging and LC3 levels showed CAPS localized to and inhibited lysosomes. CAPS outperformed CQ in inhibiting cellular migration and invasion. *In vivo*, CAPS reduced tumor volumes by 50% ($P < 0.001$) and decreased liver metastasis ($P=0.04$). CAPS displayed a 10-20%(wt/wt%) OX-loading capacity. *In vitro* testing showed a synergistic enhancement in the cytotoxicity of Chemo-CAPS that was similar to the combination of CQ and OX. **Conclusion**: CAPS displayed the biological activity of CQ to inhibit autophagy, but a greater ability to reduce PC migration and invasion *in vitro* and tumor growth and metastasis *in vivo*. These findings suggest that CAPS improves tumor accumulation. Chemo-CAPS encapsulates high levels of OX and synergistically improves cytotoxicity *in vitro*. Future studies will investigate the effects of Chemo-CAPS on tumor growth and metastasis and tumor levels of CQ and OX.

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Heterozygous Loss-of-function Mutations in Angiopoietin1 Is Associated with Primary Congenital Glaucoma

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Afflicting 60 million patients worldwide and leaving 8 million sightless, glaucoma is a devastating disease with no cure. Primary congenital glaucoma (PCG) is a particularly severe form of the disease with severe defects in ocular fluid drainage structures. We recently identified the *TEK* gene, encoding an endothelial specific receptor type tyrosine kinase, as a PCG causative gene. Genetic loss of angiopoietin-TEK signaling in mice led to severe defects in the development of ocular fluid drainage structures, resulting in congenital glaucoma. In this study, we report the identification of novel mutations in angiopoietin1 (*ANGPT1*), a major agonistic ligand for TEK receptor, in an international PCG cohort. We also report the mechanism whereby ANGPT1 function is lost in the mutant and functional impact of mutations on Schlemm's canal formation, a major ocular fluid drainage structure. **Methods:** To identify novel PCG-associated genes, we sequenced the *ANGPT1* gene in an international cohort of PCG families without other known causative gene mutations. To determine the potential functional significance of the identified *ANGPT1* mutations, we examined cellular secretion and multimerization patterns of ANGPT1 proteins carrying these mutations. Specifically, cDNA encoding individual ANGPT1 mutants, alongside with the wild-type control, were subjected to cell transfection studies followed by immunoblotting and immunocytochemistry. To explore the functional role(s) of ANGPT1 in outflow tract development, we analyzed *Angpt1* conditional knockout mice (*Angpt1* cKO). **Results:** We have identified two heterozygous novel *ANGPT1* variants (p.Q236X and p.R494X) in patients with PCG. These two mutations showed complete functional loss and possible dominant negative effects in cell-based assays. The p.Q236X mutant lacks the receptor binding domain, but retains its homo-oligomerization domains. This mutant bound to wild-type ANGPT1 and incorporated into the ANGPT1 multimers. Another mutant p.R494X only lacks terminal 5 amino-acids outside the receptor binding surface. Surprisingly, immunocytochemical analyses showed that p.R494X mutant was not secreted but sequestered inside the cells, trapping the wild-type ANGPT1 protein. In keeping with the human genetic and cell findings, histological analyses of conditional knockout mice identified a dominant role of ANGPT1 for Schlemms's canal development; *Angpt1* cKO mice exhibited severe hypomorphic Schlemm's canal, contributing to elevation of intraocular pressure. **Conclusions:** Our results provide evidence that the ANGPT1 mutations identified in primary congenital glaucoma patients are loss-of-function with possible dominant negative effects. ANGPT1 mutations may account for the pathogenesis of this devastating disease.

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Neutrophil Factor XII Regulates Cell Function and Thromboinflammation

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Deep vein thrombosis and pulmonary embolism constitute a leading cause of cardiovascular death. New anticoagulation therapies have been developed, however these therapeutic advances are still associated with increased rate of bleeding. This highlights the urgent need for the development of safer strategies to treat VTE. In addition, recent evidence indicates that inflammatory processes and VTE are closely linked. Factor XII (FXII) deficiency is associated with decreased neutrophil function the mechanisms, however, are not characterized. Our goal was to examine how FXII contributes to thromboinflammatory diseases with the aim to recognize targets for innovative therapeutics. **Methods and results:** Our studies indicate that FXII deficient (*F12*$^{-/-}$) mice have significantly lower neutrophil recruitment in two models of sterile inflammation [skin wounds and thioglycolate-induced peritonitis] and reduced venous thrombosis. To determine if FXII directly contributes to neutrophil function, we used *F12* siRNA to target FXII production in the liver, currently the only known site of FXII synthesis. Kinetic experiments determined that *F12* siRNA treatment reduced plasma *FXII coagulant activity* to < 5% within 24 h. Interestingly unlike *F12*$^{-/-}$ mice, *F12* siRNA-treated mice did not exhibit a significant reduction in neutrophil recruitment. Infusion of human FXII into *F12*$^{-/-}$ mice did not correct cell migration into the peritoneum. We next determined if FXII is expressed in neutrophils. We found evidence of *F12* cDNA in murine and human neutrophils. When neutrophils are activated with fMLP, FXII antigen translocates to the external membrane and is eventually secreted in the supernatant. We delineate that autocrine FXII signals in neutrophils through the urokinase plasminogen activator receptor (uPAR) promoting pAkt2S^{474}, a key mediator of integrin activation, neutrophil migration and neutrophil extracellular traps (NETs) formation. Functional and microfluidic assays confirm that FXII promotes neutrophil adhesion and is a potent chemotaxin. These effects on neutrophils are independent of its enzymatic function. Adoptive bone marrow (BM) transplantation (BMT) experiments show that WT BM transplanted into KO hosts corrects the leukocyte migration defect. Inferior vena cava (IVC) thrombosis induced by 90% restriction to flow at 24 h results in smaller thrombi in *F12*$^{-/-}$ than WT mice (pF12$^{-/-}$ hosts corrects the thrombus weight and degree of inflammation in *F12*$^{-/-}$ mice to normal. Plasma FXII alone is unable to fully correct thrombus weight and the inflammatory response in *F12*$^{-/-}$ mice. **Conclusions.** These studies indicate that FXII has a sophisticated role in thromboinflammatory disorders. Neutrophil-derived FXII functions upstream of hepatic FXII where it contributes to initial neutrophil activation and trafficking at sites of inflammation and venous stasis. At these sites, neutrophils support thrombus growth by hepatic FXII. These studies improve our understanding on the contribution of neutrophils in sterile thromboinflammatory states and introduce a scientifically sound approach to targeting these diseases.

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A Novel *CD34*/*ETO2*/*IFNGR* Gene Regulatory Axis is Implicated in Poor-prognosis Cases of t(8;21) Acute Myeloid Leukemia

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Roughly 12% of acute myeloid leukemia (AML) cases are derived from the t(8;21) translocation, a molecular event that results in the production of the AML1-ETO (AE) fusion transcription factor. This aberrant protein induces a broad dysregulation of the transcriptome and causes expansion of leukemia stem cells (LSCs), which predisposes clones to an increased risk of second-hit mutations. A number of LSC characteristics have been shown to influence patient survival, including expression levels of the cell surface glycoprotein, CD34, and of the truncated isoform of AE, AML1-ETO9a (AE9a). Despite these findings, a unified model of how these factors interact to further the progression of t(8;21) AML has not been determined. **Results:** Using a series of gene knockdown and knock-in experiments, we observed a causal, positive relationship between AE/AE9a and CD34 expression. This prompted us to analyze previous ChIP-seq data from Kasumi-1 cells, and we discovered high levels of AE binding to an upstream enhancer of *CD34*. Next, we used differential gene expression analyses to determine the CD34-coexpressed genes from a publically available patient microarray dataset (GSE14468, n=526). CD34-high patient samples showed an accompanying enrichment of *JUP, KIT, CD133, HEB, E2A* and *ETO2*, and a decrease in immune signaling molecules such as *IFNGR1* and *IFNGR2*. ETO2 is the only ETO family member significantly expressed in HSCs and is an important corepressor of E-proteins HEB and E2A; it is thought to help repress the pro-differentiation and anti-proliferation effects of E-proteins. ETO2, being an ETO family member, is also able to oligomerize with other ETO family members, such as ETO, and thus may play a role in modifying the functions of the AE-transcriptional complex. Intriguingly, we have shown that AE and AE9a repress *ETO2*, although AE9a does so to a lessened extent. Finally, we performed Kaplan-Meier analyses with the microarray data (a majority of the patient samples included prognostic metadata). We found a significant relationship between high ETO2 expression or low IFNGR expression and lower survival. **Conclusions:** Our findings suggest a clinically relevant association between CD34, ETO2, and IFNGR in both t(8;21) and non-CBF AML. We believe this regulatory axis is especially relevant in relapse likelihood and may be hijacked by LSCs to preserve their “stemness” and resistance to therapy. Our findings may suggest that AE simultaneously activates *CD34* and represses *ETO2*, providing explanation for why full-length AE is slow to induce leukemia in mouse models and a relatively good prognostic mark in humans. Additionally, our findings suggest a loss of *ETO2* repression as a potential mechanism by which the AE9a isoform, and perhaps other second-hit mutations, converts LSCs to a more dangerous phenotype.

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Atrial-specific Deletion of AMP-activated Protein Kinase (AMPK) Disrupts Normal Electrical Gene Programming, Culminating in the Development of Atrial Fibrillation (AF)

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Aims: To elucidate the role of AMPK in maintaining atrial electrical homeostasis and examine the molecular mechanisms linking AMPK deletion and AF development. **Background:** AF is a major health problem. Current treatment is limited to anti-arrhythmic drugs and ablation, which have modest efficacy and side effects. Our hypothesis that AMPK has a critical role in atrial biology and arrhythmias is based on recent human and experimental studies. Biopsy results suggest that AMPK activation is altered in human AF. Global cardiac deletion of AMPK and its upstream activator, liver kinase B1, in the mouse leads to heart failure and spontaneous AF. To avoid the confounding ventricular dysfunction, we have developed a novel genetic model with atrial-specific deletion of AMPK to study the autonomous role of AMPK in the atria. **Methods:** To generate atrial-specific AMPK double-knockout (dKO) mice, we crossed mice with floxed AMPK α1 and α2 isoform genes with mice expressing Cre-recombinase from the sarcolipin promoter. Cardiac function, structure, and electrophysiology were studied using ECG, echocardiography, immunohistochemistry, and qPCR. To determine the molecular effects of atrial AMPK deletion, we utilized siRNA knockdown in primary neonatal atrial myocytes. **Results:** AMPK-dKO mice displayed P-wave prolongation on ECG (16.6 vs. 7.7 ms, $p < 0.001$) at 1 week, indicating intra-atrial conduction delay. Atrial ectopic complexes, potential triggers of AF, were common in AMPK-dKOs. QRS complexes were normal. AMPK-dKO mice developed spontaneous AF at 6 weeks, in the absence of structural changes (normal echocardiograms, histology, and hydroxyproline content). However, determinants of intra-atrial conduction, including ion channel and gap junction protein (GJP) transcripts, were altered in 1-week-old right (RA) and left atrium (LA). *SCN5A* (voltage-gated sodium channel 1.5) showed a 70% reduction in RA and LA, while the atrial-selective connexin-40 displayed a 50% decrease in LA (all $p < 0.001$). Further, *HCN4*, gene encoding funny current channel that promotes atrial pacemaker activity, was increased by twofold in LA ($p < 0.05$). These changes were observed in the setting of downregulation of *PITX2C* (0.42 vs. 1.00, $p < 0.001$), the major left atrial transcription factor responsible for left-right atrial asymmetry. siRNA knockdowns of *PRKAA1* and *PRKAA2* (α1 and α2 catalytic subunits) in neonatal left atrial myocytes resulted in a significant decrease in *PITX2C* and increase in *HCN4* transcript levels. **Conclusions:** We developed a novel genetic approach to specifically study atrial arrhythmogenesis. Results demonstrate that the metabolic fuel gauge AMPK has an autonomous effect in the left atrium to regulate ion channels and GJPs that are important determinants of intra-atrial conduction and arrhythmogenesis. AMPK specifically regulates the left atrial transcription factor Pitx2c and downstream targets, including *HCN4*, which may promote atrial ectopy and trigger AF. Our results provide the foundation to study pharmacological AMPK activation as a novel therapy for the prevention and treatment of AF.

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The Transcription Factor Ets1 Cooperates with IL17 Signaling to Promote Autoimmunity and Immunodeficiency

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Ets1 is a transcription factor that serves as a negative regulator of plasma cell formation. Mice that lack Ets1 (Ets1 KO) develop spontaneous autoimmune disease with high levels of autoantibodies and formation of immune complexes. Th17 cells are a type of CD4+ T helper cell that produce the cytokine IL-17. Th17 cells and IL-17 are strongly implicated in the pathogenesis of multiple autoimmune diseases. It has been previously reported that naïve CD4+ T cells isolated from Ets1 KO mice have an increased propensity to differentiate into Th17 cells and produce IL-17. In order to determine if increased IL-17 production contributes to the development of autoimmunity in Ets1 KO mice, our lab created a combined Ets1/IL-17 Receptor double knock out (DKO) model. Unexpectedly we found that the loss of IL-17 Receptor did not prevent the development of autoimmune disease, but rather enhanced it. Furthermore, the DKO mice develop spontaneous skin lesions and display an increased susceptibility to skin infections from the pathogen *Staphylococcus aureus* (*S. aureus*). Curiously, the increased susceptibility to *S. aureus* skin infections seems to be age-dependent as 2 month old DKO mice do not show an increased susceptibility, whereas 4 month old DKO mice do. We are currently looking into mechanisms that could explain the immunodeficient phenotype of DKO mice. We found that DKO mice have many more effector T cells that can provide help to B cells in antibody production (T helper 2 cells and T follicular helper cells), which is associated with a massive increase in the number of germinal center B cells, memory B cells and plasma cells. DKO mice also have increased B cell isotype-switching to IgG1 and IgE. This immune activation is associated with the development of autoantibodies against antimicrobial peptides that are known to be important in controlling bacterial infections. Our current model is that excessive immune activation over time leads to B cell production of autoantibodies that target immune effector mechanisms important for anti-bacterial immunity, resulting in susceptibility to *S. aureus*. In some human diseases like Wiskott–Aldrich syndrome (WAS) or chronic granulomatous disease (CGD), both autoimmunity and immunodeficiency are present at the same time, similar to what is seen in DKO mice. Therefore, our studies could have important implications in understanding the mechanisms that drive pathogenesis in these types of human diseases characterized by both autoimmunity and immunodeficiency.

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An Activating Mutation of the NSD2 Histone Methyltransferase Drives Oncogenic Reprogramming in Acute Lymphocytic Leukemia

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Acute lymphocytic leukemia (ALL) is a disease of immature lymphocytes that overgrow their niche and impede normal hematopoiesis, resulting in death without definitive therapy. While significant strides have been made in the treatment of ALL, relapse remains a crucial concern, and ALL remains the most common cause of cancer-related death in children. Recent sequencing advances have revealed mutations in genes encoding epigenetic regulators, particularly enzymes that read, write, and erase chemical modifications on histone tails, frequently occur in relapsed ALL and other malignancies. The histone methyltransferase, *NSD2*, is a histone lysine methyltransferase whose overexpression plays a significant role in multiple myeloma. Recently, a glutamic acid to lysine point mutation (E1099K) in NSD2 was discovered in relapsed ALL and other cancers. This mutation appears to activate NSD2 function, increasing histone H3 lysine 36 dimethylation that is associated permissive transcription and an oncogenic phenotype. We hypothesize that the NSD2 E1099K point mutation alters its chromatin-binding properties, driving widespread epigenetic reprogramming that promotes cancer progression. **Methods**: To test how the E1099K mutation may alter binding of NSD2 to chromatin, we transfected 293 cells with GFP-tagged wildtype (WT) or mutant NSD2 and measured diffusion dynamics by fluorescence recovery after photobleaching (FRAP). To measure the role of E1099K on ALL biology we used CRISPR/Cas9 to generate isogenic ALL cell lines differing only in the status of the E1099K mutation in NSD2 and performed RNA-seq, adhesion, proliferation, colony formation and xenograft assays. **Results:** FRAP demonstrated that cells expressing GFP-tagged NSD2 E1099K recover fluorescence intensity at a slower rate than cells expressing GFP-tagged WT NSD2. In addition, the mobile fraction of GFP-tagged NSD2 E1099K was significantly less than that of GFP-tagged WT NSD2, suggesting that mutant NSD2 binds more tightly to nucleosomes than WT NSD2. Transcriptional profiling of CRISPR-edited cells revealed that, in ALL cells harboring NSD2-E1099K, genes involved in cancer-associated pathways, adhesion programs, and neural lineage were strongly upregulated. Accordingly, E1099K-positive cells displayed enhanced proliferation, colony formation, adhesion, and migration, while mice xenografted with E1099K-positive ALL cells succumbed to pervasive disease and hind-limb paralysis significantly faster than their wildtype-NSD2 counterparts. Tracking the cells by *in vivo* imaging revealed that the NSD2 E1099K cells preferentially migrated to the head, suggesting a potential cause of death. **Conclusions:** These results suggest that the increased activity of mutant NSD2 may be due to tighter binding to chromatin compared to WT, causing off-target binding of NSD2 and spread of H3K36me2. Using CRISPR-edited cells with wildtype or mutant NSD2, we demonstrate that NSD2-E1099K drives a transcriptional program that enhances oncogenic properties in ALL. We establish that targeting this mutant enzyme and its ability to bind and methylate histones could be a valuable therapy in relapsed ALL and other related malignancies.

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Myeloid Krüppel-like Factor 2 Deficiency Contributes to Metabolic Syndrome Via c-Jun N-terminal Kinase-mediated Inflammasome Activation

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Metabolic syndrome is a constellation of clinical findings consisting of obesity and hyperglycemia that considerably increases the risk of developing type II diabetes and cardiovascular disease. With a prevalence of nearly 33 percent in the United States, metabolic syndrome poses a significant burden on the healthcare system and the quality of life of patients. Current evidence suggests that macrophage-mediated inflammation fosters adiposity and insulin resistance associated with metabolic syndrome; however central regulators of this process remain elusive. One current explanation involves activation of the inflammasome. Originally described in the context of infection, the inflammasome is an oligomeric protein complex with the ultimate function of producing activated interleukin-1β (IL1β), a potent inflammatory cytokine. Inflammasome activation is a highly regulated process involving both transcriptional and post-translational control. Previous work in our group has identified Krüppel-like factor 2 (KLF2) as a tonic repressor of macrophage activation in the context of infection and autoimmune disease. Release of this ⍰transcriptional brake⍰ via genetic knockout leads to enhanced inflammatory potential for macrophages, providing the basis of our hypothesis that deficiency in myeloid KLF2 is capable of manifesting metabolic syndrome via increased macrophage activation. Interestingly, mice harboring myeloid-specific deletion of KLF2 (K2KO) exhibit increases in body weight on either a normal or high-fat diet compared to control mice. After one month of high-fat diet, these mice also display a phenotype that is consistent with metabolic syndrome, including leptin and insulin resistance, increased adiposity, and inflammation of adipose, liver, and skeletal muscle. Additionally, overexpression of KLF2 in the myeloid line conferred resistance to many of the detrimental outcomes of high-fat diet. Mechanistically, there is a substantial increase in inflammasome oligomerization and activity in K2KO macrophages. To describe this activation, we turned to the known post-translational activator of inflammasome oligomerization, c-Jun N-terminal kinase (pNK). K2KO macrophages have significantly higher levels of activated JNK (pJNK), which has been implicated in both inflammasome oligomerization and metabolic syndrome. Additionally, K2KO mice have higher pJNK activity in their adipose tissue compared to control mice. These data establish KLF2 as a critical regulator of macrophage activation during the genesis of metabolic syndrome via a novel KLF2-JNK-inflammasome axis. These findings will serve as a nidus for future studies in targeted therapies for metabolic syndrome.

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Characterization of Novel Mediators of Pre-metastatic Niche Formation

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Metastasis is the leading cause of cancer-related mortality. This multistep process requires that disseminated tumor cells (DTCs) evade immune surveillance and adapt to a hostile environment in the metastatic organ. Evidence from mouse models and human samples shows that the primary tumor can orchestrate the production of pro-inflammatory molecules and infiltration of bone marrow-derived myeloid cells in the metastatic organs before the arrival of cancer cells, thus creating a pre-metastatic niche. It has been shown that the pre-metastatic niche is essential for the success of the metastatic process. However, the exact mechanism how the primary tumor promotes the formation of this niche and the exact step in the metastatic cascade where this process is crucial, are largely unknown. To address this question we use an orthotopic model of stage IV triple negative breast cancer that preferentially metastasizes to the lungs (4T1). We observed that after injection of a primary tumor neutrophils and inflammatory monocytes progressively accumulate in the lungs and blood. As the number of myeloid progenitors increased in the bone marrow, we think that the primary tumor produces factors that impact on myelopoiesis. Surgical removal of the primary tumor completely abolished the changes in the immune infiltrate of the lungs proving that those modifications are primary tumor-dependent. Moreover, removal of the primary tumor at different times allowed us to trace back the timepoint when cells start to migrate from the primary tumor. In addition, we are currently developing a model for dormant breast cancer metastasis, where orthotopically injected 4T07 cells tumor cells migrate to the lungs but do not produce macroscopic metastasis. Our future plans include to further characterize this model and compare the difference in the immune infiltrate of the lungs in the intra and extra-vascular compartments of both models. The comparison of 4T1 and 4T07 will enable us to further dissect whether the pre-metastatic niche is necessary only for the initial seeding of tumor cells or whether it is also necessary to the proliferation and survival of the DTCs in the target organ. Once both models are sufficiently characterized, we will choose a time point when tumor cells are not yet detectable in the lungs and perform an unbiased analysis of transcripts and micro-RNAs. We expect to identify novel pathways of pre-metastatic niche formation that can be therapeutically relevant. In follow-up experiments, we will interfere with those selected pathways to try to prevent metastasis formation.

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Epigenetic Pathways Regulating HOTAIR Expression in Glioblastoma.

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Glioblastoma multiforme (GBM) is the most common and aggressive malignant adult brain tumor. Even with surgical resection followed by radiotherapy and chemotherapy, the 5-year survival rate is approximately 10%. Therefore, there is an urgent need for novel therapeutic development. Histone deacetylase inhibitors (HDACi) have emerged as promising therapeutic agents in GBM. HDACi are small molecules that interfere with histone tail modification, thus altering chromatin structure and epigenetic pathways. A long non-coding RNA termed HOTAIR (HOX transcript antisense RNA), is an important epigenetic regulator with critical roles in GBM tumor cell proliferation. However, mechanistic into HDAC dependent control of HOTAIR expression has remained elusive. We hypothesized that bromodomain-containing protein 4 (BRD4) that binds to HOTAIR promoter could provide insight into regulation of HOTAIR transcription by HDACs. **Methods:** We used the LN18 cell line to screen a library of 61 compounds acting as inhibitors of epigenetic enzymes. The dose selective action of HDACis on HOTAIR reduction was tested on 2 human patient-derived glioblastoma cells (PDX22 and PDX76). We extracted RNA, measured HOTAIR expression via qRT-PCR and analyzed the levels of proteins controlling HOTAIR expression including BRD4 via Western blot analysis. shRNA constructs targeting individual HDACs (1-11) were used to knockdown HDAC in PDX22 cells. To better understand the mechanism, chromatin immunoprecipitation was done to assess whether HDACi prevent BRD4 binding to the HOTAIR promoter. **Results:** From the screening of an epigenetic drug library, Tricostatin A, NSC-3852, BML-281, Apicidin, M-344, Scriptaid, Oxamflatin and Vorinostat (SAHA) reduced HOTAIR levels more than 50%. Treatment of the patient-derived glioblastoma cells and the U87-MG cell line with HDACi resulted in a dose-dependent decrease in HOTAIR RNA expression. Furthermore, HDACi results in downstream degradation of BRD4 and redistribute BRD4 from the HOTAIR promoter, suggesting that HDACs normally regulate BRD4 localization to the HOTAIR promoter. **Conclusion:** Our data unravels a previously unappreciated mechanism through which HDAC inhibition modulate GBM tumor cells growth by reducing the recruitment of BRD4 to the HOTAIR promoter. Importantly, these results suggest that HOTAIR may be a biomarker for responsiveness of GBM and other cancers to HDAC inhibitors.

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Defining the Role of Cancer-associated Mutations of the Protein Phosphatase 2A A-alpha Subunit in Endometrial Carcinomagenesis

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Protein phosphatase 2A (PP2A) is a major serine-threonine phosphatase that directly regulates many diverse and essential cellular pathways. PP2A is a heterotrimeric enzyme composed of a scaffolding A-subunit, catalytic C-subunit, and one of several possible regulatory B-subunits. Study of PP2A in cancer has identified its role as a tumor suppressor. Analysis of genomic data from human tumors revealed somatic mutations that cluster at a high frequency to key interaction points of the Aα-subunit, the predominant scaffolding subunit isoform, with its regulatory B-subunits. Of note, uterine endometrial cancers accounted for nearly 50% of identified Aα mutations, while a subset, including the mutations P179R and S256F, were further identified as uniquely specific to cancer of the endometrium (TCGA). To date, the potential pathogenic and disease-specific role of these mutations in endometrial carcinoma has not been fully elucidated. However, their specificity, recurrence, and location at structurally important sites suggest a biological impact on PP2A assembly and function that may be advantageous to cancer development. Preliminary work has demonstrated significant structural alteration to the mutant P179R-Aα protein, which is coupled with a nearly global loss of binding to regulatory B-subunits, thereby disrupting holoenzyme assembly. Ongoing research aims to characterize how PP2A-Aα mutations may contribute to endometrial carcinomagenesis, tumor growth, and alteration of PP2A-regulated signaling dynamics.

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Development of a Novel Single-Session Cardiac Positron Emission Tomography Protocol to Evaluate Post-Infarction Inflammation and Metabolic Viability in a Porcine Model of Acute Myocardial Infarction

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Static cardiac positron-emission tomography (PET) imaging with ^{18}F-fluorodeoxyglucose (FDG) has traditionally been used to evaluate myocardial viability under non-fasting conditions by visualizing metabolic defects in the heart. Recent studies suggest that FDG PET imaging performed under fasting conditions may also provide prognostic value by allowing visualization of glycolytic inflammatory processes, such as sarcoidosis or infarct healing. The present study was conducted to determine whether fasting and non-fasting (i.e., insulin-stimulated) FDG PET imaging could be combined in a single imaging session to evaluate myocardial inflammation and metabolic viability using Patlak kinetic analysis of cardiac FDG uptake under two different kinetic states. **Methods:** Closed-chest, propofol-anesthetized swine (n=9) were subjected to a 1 hour mid-LAD occlusion to produce myocardial infarction. Five days later, list-mode cardiac FDG PET was performed for 20 minutes following an overnight fast with intravenous heparin to suppress FDG uptake in remote myocardium. Next, an Insulin/Dextrose clamp was produced by administering an insulin bolus followed by a continuous infusion of insulin and dextrose. A second FDG dose was administered and the scan was repeated. Based on Patlak kinetic analysis and blood glucose levels, the influx constant Ki (mL tissue/L blood*min) was converted to regional myocardial glucose uptake (rMGU, μmol/100g^{-1}*min^{-1}). **Results:** Myocardial glucose uptake was significantly increased in the infarct region compared to remote myocardium, under fasting conditions (11.5 ± 1.1 vs 3.4 ± 0.8 μmol glucose/100g^{-1}*min^{-1}, $p < 0.01$), consistent with the presence of glycolytic inflammatory cells 5-days after myocardial infarction. Insulin stimulation significantly increased rMGU in remote myocardium (10.5 ± 1.8 vs 3.4 ± 0.8 μmol glucose/100g^{-1}*min^{-1}, $p < 0.01$), but not infarcted myocardium (14.8 ± 1.2 vs 11.5 ± 1.1 μmol glucose/100g^{-1}*min^{-1}, p=ns), when compared to fasting conditions. Nevertheless, glucose uptake in the infarct remained greater than remote myocardium even during insulin-stimulated conditions (14.8 ± 1.2 vs 10.5 ± 1.8 μmol glucose/100g^{-1}*min^{-1}, $p < 0.05$). **Conclusions:** These results demonstrate that both fasting and insulin-stimulated cardiac FDG PET can be performed and quantified consecutively using Patlak kinetic analysis in a single imaging session. Measurement of FDG uptake during fasting conditions allows evaluation of inflammation in infarct healing, while assessment of insulin responsiveness enables evaluation of myocardial viability.

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Astrocyte-derived Extracellular Matrices have Different Growth Support Capacities depending on Astrocyte Subtype

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Every year, 12,000 Americans suffer a traumatic spinal cord injury (SCI), which often leads to some level of paralysis and has a lifetime cost between $1 and 4.5 million. One major challenge for SCI treatment is the limited regenerative capacity of the adult mammalian spinal cord. This lack of regeneration is due, in part, to astrocytes forming a highly organized glial scar that serves to limits the extent of secondary injury, but is inhibitory to new axon growth. Interestingly, it has also been observed that astrocytes are necessary for SCI recovery. These observations suggest that astrocytes play both pro-regenerative roles and inhibitory roles after SCI. We have previously derived two major spinal astrocyte subtypes, fibrous (white matter) and protoplasmic (grey matter) astrocytes, from mouse embryonic stem cells (mESCs). We found that neurons extend significantly longer axons on protoplasmic astrocyte-derived substrates than on fibrous astrocyte-derived substrates. This led us to harvest the ECM deposited by both protoplasmic and fibrous astrocytes to test whether astrocyte ECM incorporation improves neurite growth on injectable hyaluronic acid (HA) hydrogels. Since decellularized, xenogenic ECM materials have been successfully transplanted without requiring immuno-suppression, we hypothesize that these HA-ECM materials can be transplanted into a rat SCI model and improve histological recovery without requiring immunosuppression (as is needed for live xenogenic cells). **Methods:** Both astrocyte subtypes were derived from RW4 mESCs using bone morphogenetic protein 4 to generate protoplasmic astrocytes and ciliary neurotrophic factor (CNTF) to generate fibrous astrocytes. After 6 days of culture, astrocyte plates were decellularized, and the ECM harvested and lyophilized with 50 mM trehalose. For hydrogels: HA-furan was mixed with various weight

ratios of ECM along with PEG-dimaleimide so that a hydrogel formed. The ability of the resulting gels to support neurons was assessed using pure mESC-derived motor neuron cultures. **Results:** We found that ECM incorporation improved neurite outgrowth on HA hydrogels *in vitro*. We observed that neurite growth improved as ECM concentration increased, and that protoplasmic ECM was more potent than the fibrous ECM, consistent with our studies on decellularized ECM alone. We transplanted hydrogels conjugated with Alexa Fluor® 555 without ECM incorporation into rats following SCI and could detect the hydrogels for up to 2 weeks following transplant. This demonstrates that these HA gels have sufficient stability for use as a SCI treatment. **Conclusions:** These results demonstrate that protoplasmic astrocyte-derived ECM harvested from tissue culture maintains neuronal growth benefit and that the neurite growth permissive properties of this ECM is intrinsic to the deposited components. Overall, HA-ECM hydrogels could represent a novel approach for improving regeneration following SCI. These gels have many useful properties for translation, including: the ready capacity of ESCs for expansion, easy storage, and injectability.

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ERP Signatures of Environmental Sound Processing in Language Context

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Despite the fact that environmental sounds like gun shots and dog barks are very different from words acoustically, and bear a very different relationship to meaning and concepts, we have found that they are quickly and easily processed in sentence context. In two previous behavioral studies, sentences ending in environmental sounds or in spoken words showed similar effects of sentence constraint on recognition and understanding. This suggests that listeners can understand the meaning of environmental sounds and use linguistic context to aid in understanding these sounds in much the same way as for spoken words. However, similar patterns of behavior can occur from different neural mechanisms. The present study was carried out to investigate the underlying neural processes that mediate the understanding of sound patterns and speech. We conducted an ERP study in which listeners heard sentences ending in either an environmental sound or a spoken word. In half the sentences, the last item made sense with the preceding context ("sense"), and in half it did not ("nonsense"). Additionally, half the sentence stems were specific (high constraint i.e. strongly suggested the last item) and half were general (i.e. low constraint). We used topographic bootstrapping methods to test for significant scalp topography differences between conditions during the time period after last item onset. Environmental sounds in sentence context elicited a strong N1-P2 complex that was not seen for spoken words; in other studies, the N1-P2 is related to auditory plasticity. Nonsense sentences ending in words produced a stronger central negativity corresponding to the typical N400 than meaningful sentences; this result confirms previous research on the N400. The N400 is thought to index recognition or integration processes that are more difficult for nonsensical stimuli. By comparison, environmental sounds elicited two phasic ERP responses with similar central-negative scalp distribution in the same N400 time window. The earlier varied with meaningfulness, with a more central-negative topography for nonsense sentences; the second varied with sentence constraint, with a larger response for general (rather than specific) sentence frames. The fact that the N400, typically higher-amplitude for nonsense sentences, is not higher-amplitude for sounds than for words suggests that listeners do not treat the sounds, when in a meaningful context, as nonsense on the surface. Our data further suggest that similar behavioral patterns arise from different underlying neural processing, which may reflect relative unfamiliarity with recognizing environmental sounds for explicit meaning, as well as with hearing these sounds in the context of a spoken sentence.

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DNMT3A Is a Haploinsufficient Tumor Suppressor in Murine CD8$^+$ Peripheral T-Cell Lymphoma

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DNA methyltransferase 3A (DNMT3A) is a master epigenetic regulator of benign and malignant hematopoiesis. DNMT3A loss of function, either through gene deficiency or dominant negative point mutation, is the initiating event in the development of myriad human hematologic malignancies. Interestingly, either homozygous or heterozygous mutations can occur in these neoplasms – the frequency of which varies by hematopoietic lineage. In human Dnmt3a-mutated malignancies, myeloid neoplasms almost exclusively harbor heterozygous mutations whereas mutations in lymphoid neoplasms are predominately, but not exclusively, homozygous. Thus, it is unknown as to whether lesion of one or two Dnmt3a alleles is necessary for inciting malignancy. To dissect the biological consequences of homozygous and heterozygous Dnmt3a mutations in malignant hematopoiesis, we generated Dnmt3a homozygous null (*Dnmt3a*$^{\Delta/\Delta}$) and Dnmt3a heterozygous deficient (*Dnmt3a*$^{+/-}$) mice and compared the presentations of hematologic malignancies between cohorts. Bi-allelic inactivation of Dnmt3a results in the presentation of mature lymphoid neoplasms resembling chronic lymphocytic leukemia (CLL; B220$^+$CD19$^+$CD5$^+$; 88% penetrance) and CD8-positive peripheral T-cell lymphoma (PTCL; TCRβ$^+$CD3$^+$CD8$^+$CD4$^-$; 41%). In contrast, mono-allelic inactivation of Dnmt3a results in the presentation of CLL and PTCL at reduced penetrance (47% & 10%) and, rarely, a mature myeloproliferative neoplasm (MPN; CD11b$^+$Gr-1$^+$; 10%). As PTCL is a rare and understudied class of lymphoma lacking genetically relevant animal models, we chose to molecularly characterize *Dnmt3a*$^{\Delta/\Delta}$ and *Dnmt3a*$^{+/-}$ tumors by RNA-seq, WGBS, and targeted cDNA sequencing. Tumors displayed genome-wide deregulation of DNA methylation, characterized by 10-fold greater hypomethylation than hypermethylation of promoters and enhancers. Transcription factor binding sites for AML1, NF-κB, and OCT1 were enriched in hypomethylated promoters, implicating these transcription factors in tumor pathogenesis and/or Dnmt3a-associated DNA methylation. Whereas 71 hypomethylated genes showed an increased expression in PTCL, only 3 hypermethylated genes were silenced, suggesting that cancer-specific hypomethylation more frequently affects the transcriptome than hypermethylation in lymphoma. Transcriptomes of *Dnmt3a*$^{+/-}$ and *Dnmt3a*$^{\Delta/\Delta}$ lymphomas were largely conserved between genotypes and significantly overlapped with those of human tumors. Importantly, we observed downregulation of p53 in *Dnmt3a*$^{+/-}$ and *Dnmt3a*$^{\Delta/\Delta}$ lymphomas. Repression of p53 protein occurred as well in pre-tumor thymocytes of 9 months old, but not 6 weeks old, *Dnmt3a*$^{+/-}$ disease-free mice, suggesting that p53 downregulation is an intermediate event in

tumorigenesis. DNA sequencing, RNA-seq, and WB analysis of *Dnmt3a*$^{+/-}$ tumors revealed that PTCL develops without mutation or silencing of the remaining wild-type allele, indicating that Dnmt3a is a haploinsufficient tumor suppressor in T-cell lymphomas. Analysis of known coincident human PTCL drivers, *RhoA* and *Tet2*, did not reveal any coding mutations or significant transcriptional changes in *Dnmt3a*$^{+/-}$ or *Dnmt3a*$^{\Delta/\Delta}$ lymphomas. These data demonstrate that in mice, Dnmt3a is a haploinsufficient tumor suppressor in mature CD8^{+} PTCL and downregulation of the tumor suppressor p53 occurs as an intermediate event in tumorigenesis.

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Analysis of Hypoxia-related Gene Expression in Head and Neck Cancer

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Tumor hypoxia is associated with specific alterations in gene expression, including upregulation of hypoxia-inducible factor (HIF) targets. In some contexts, including malignant glioma, tumor hypoxia and expression of hypoxic response genes (HRGs) is thought to promote resistance to anticancer therapies, leading to poorer survival. Recently published data suggests HRG expression in malignant glioma may be associated with increasing age of tumor cells of origin, potentially contributing to the worse prognosis observed amongst elderly patients with this disease, but it is unclear whether similar mechanisms may exist in other cancer types. Using data from The Cancer Genome Atlas, we observed an association between expression of a hypoxic response set of 86 HIF-targeted genes and patient survival in head and neck squamous cell carcinoma (HNSC). In contrast to results reported for malignant glioma, there was minimal overall association between expression of the HRGs and patient age in HNSC. Therefore, we further analyzed the hypoxic response set in HNSC to characterize relevant subsets and biological pathways. Interestingly, we observed a particularly strong correlation between a four gene set composed of COL5A1, FN1, MMP2, and MMP14, all genes associated with the extracellular matrix (ECM), and the overall behavior of the HRG set. Expression of these ECM-related genes was negatively associated with patient survival. These data suggest hypoxia-related effects on the ECM may influence outcomes in HNSC.

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Ambient Air Pollution Particulate Matter Impairs Airway Antimicrobial Peptide Activity

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Sustained exposure to ambient air pollution is a global cause of mortality due to respiratory illnesses. Particulate matter (PM) is a major constituent of ambient air pollution capable of causing airway disease. The lungs are lined with fluid called Airway Surface Liquid (ASL), which contains antimicrobial proteins and peptides (AMPs) constituting one of the first lines of defense against invading bacteria. Particles and proteins can interact, altering their structure and function. **Objectives:** We hypothesize that PM can interact with ASL AMPs and impair their antimicrobial activity. **Methods:** We assessed the antimicrobial activity of samples exposed to PM, specifically: pig and human airway explants, pig and human ASL, and the cationic airway AMPs human beta defensin-3, LL-37, and lysozyme. We measured the adsorption of AMPs by PM, surface charge of PM, and the AMPs exposed to PM. **Results:** We found increased bacterial survival in PM exposed airway samples. In addition, we report that PM samples studied had a negative surface charge and adsorb cationic AMPs to form negative particle-protein complexes. When we treated PM with ethylene glycol tetraacetic acid (EGTA), we found a less negative surface charge, decreased adsorption of AMPs, and prevention of the airway antimicrobial activity impairment caused by PM. **Conclusion:** We propose that, when PM arrives at the airway, it rapidly adsorbs AMPs and creates negative complexes, thus decreasing the functional amount of AMP and making the airways more susceptible to bacterial infections. These results provide a novel translational insight into an early mechanism of ambient PM induced respiratory infections.

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Type 2 Inflammatory Responses Protect Against Staphylococcus aureus-induced Sepsis

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According to the CDC, methicillin-resistant *Staphylococcus aureus* (*S. aureus)* infections account for nearly 1% of all hospitalizations (>300,000) in the United States. Recent studies indicate that type 2 cytokines, best known for their role in allergies, may be beneficial to the host during acute infection. We hypothesized that activation of adaptive type 2 responses would protect against mortality during *S. aureus* blood-stream infection. **Methods:** We tested our hypothesis using house dust-mite (HDM) sensitization and challenge to activate a type 2 response in the lungs of C57Bl/6j mice, followed by intravenous infection with a lethal dose of *S. aureus*. Lung populations were analyzed using flow cytometry. Antibody-mediated depletion was used to selectively eliminate cell populations to test if they were required for protection. **Results:** Induction of type 2 inflammation by HDM sensitization and challenge protected mice from *S. aureus*-induced mortality. The mechanism of this beneficial type 2 response against acute infection was evaluated by flow cytometry, which revealed a relative decrease in the ratio of neutrophils to eosinophils in the lungs and an increase in eosinophils in type 2-stimulated compared to untreated mice. These data suggest that the type 2 responses suppress an overwhelming neutrophilic response, which might otherwise be lethal. However, systemic depletion of neutrophils prior to infection resulted in accelerated mortality compared to non-depleted mice. This result demonstrates that neutrophils are necessary to protect against *S. aureus* infection, but does not rule out that an overabundance of neutrophils is also detrimental. Studies are underway to determine if the increased eosinophils provide protection against mortality in this model. Finally, mice with impaired type 2 responses due to deficiency in the transcription factor PLZF have accelerated mortality when infected with intravenous *S. aureus* compared with wild type mice, further supporting our hypothesis that type 2 responses are protective during acute bacterial infection. **Conclusion:** We conclude that the type 2 response is protective during acute systemic *S. aureus* infection, possibly mediated by augmented pulmonary eosinophilia as well as the activity of PLZF-dependent type 2 innate lymphocytes.

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Role of NF-kB c-Rel Transcription Factor in the Development and Cytotoxic Functions of NK Cells

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Natural killer (NK) cell development, activation, and cancer killing activity involves IL-2Rb signaling which can lead to nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) activation[1]. In light of the fact that splenocytes from mice lacking c-Rel have a profound deficiency in expression of the IL-2 cytokine[2], we sought to elucidate the role of NF-kB c-Rel transcription factor in NK cell development and cytotoxic functions. To investigate the effects of c-Rel in the development of NK cells, we initially analyzed total percentages of NK cells in the liver, spleen, and bone marrow of c-Rel$^{-/-}$ mice compared to age matched wild-type (wt) controls. We also analyzed NK cell subsets by flow cytometry as defined by CD11b^{low}, CD11b^{high}, CD27^{low} and CD27^{high} receptor expression pattern on NK1.1^{+}CD3^{-} gated cells. Finally, we examined proliferative and tumor cell killing capacity of NK cells isolated from spleen and bone marrow of c-Rel-deficient mice compared to the wild-type counterpart. We observed an increase in CD11b^{high} CD27^{low} mature NK cell subset in the bone marrow of c-Rel-deficient mice when compared to wt-controls. Despite the increase in mature NK cell subset in c-Rel-deficient mice, freshly isolated NK1.1^{+}CD3^{-} NK cells from the spleen and bone marrow of c-Rel-deficient mice are less proliferative, and exhibit decreased cytotoxic activity against 4T1 murine mammary carcinoma cells compared with NK cells from wt mice. Our data suggest that c-Rel transcription factor is important for NK cell development, proliferation and cytotoxic function. We plan to elucidate the mechanism through which c-Rel-deficiency is impairing both proliferation and cytotoxic function in NK cells.

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MiR-155 Promotes FLT3-ITD-Induced Myeloproliferative Disease Through Inhibition of Interferon Signaling

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MicroRNA expression is dysregulated in many human cancers, including hematologic malignancies. Among hematologic malignancies, acute myeloid leukemia (AML) carries a particularly poor prognosis, leading to over 10,000 deaths each year. The most common genetic aberration in AML is a gain-of-function mutation in the FMS-like tyrosine kinase 3 (FLT3) receptor. FLT3 internal tandem duplication (ITD) occurs in ~25% of all AML diagnoses, and confers a negative prognosis. MicroRNA expression has been shown to be dysregulated in FLT3-ITD+ AML, and miR-155 has been identified as the most highly overexpressed microRNA in this disease. However, the functional relevance of miR-155 in FLT3-ITD-mediated disease and the downstream effects of miR-155 expression remain unclear. **Methods and design:** In this study, we crossed mice homozygous for the FLT3-ITD mutation with miR-155 knockout mice to determine the specific role of miR-155 in the context of FLT3-ITD. The FLT3-ITD mice develop a chronic myeloid malignancy that we evaluated between 4-6 months of age, and compared this phenotype between groups (FLT3-ITD 155+/+ vs FLT3-ITD 155-/-). **Results:** FLT3-ITD miR-155-/- mice exhibited decreased myeloid expansion in the bone marrow, reduced splenomegaly, and decreased peripheral blood monocytosis and neutrophilia compared to their FLT3-ITD miR-155+/+ counterparts, indicating that miR-155 was playing a crucial role in promoting FLT3-ITD-mediated myeloproliferation. When examining the stem cell compartment of these animals, we found that miR-155 deficient animals had a reduced number of myeloid progenitors compared to their FLT3-ITD 155+/+ counterparts. This phenotype was attributed to miR-155's role in promoting proliferation, but not survival, of the hematopoietic stem and progenitor cell (HSPC) and myeloid progenitor cell compartments in the bone marrow. RNA sequencing of the HSPC population in FLT3-ITD 155+/+ mice and FLT3-ITD 155-/- mice revealed that mice lacking miR-155 had an increased response to interferon, known to have a growth-suppressive effect on hematopoietic cells. These findings were corresponded with human AML data from The Cancer Genome Atlas, where we found that FLT3-ITD+ AML samples had a decreased interferon signature compared to FLT3-WT AML samples. A number of putative miR-155 targets in the HSPCs of FLT3-ITD mice are upregulated in the absence of miR-155, including PU.1, SHIP1, and CEBPB. Interestingly, CEBPB is known to regulate interferon responses. **Conclusions and future directions:** Our study establishes miR-155 as a critical promoter of FLT3-ITD-mediated myeloproliferative disease *in vivo*, a finding we attributed to miR-155's inhibition of the interferon response in this context. Further work will determine which of miR-155's targets could be potentiating this effect. We will also focus on the importance of miR-155 in FLT3-ITD+ leukemogenesis, as FLT3-ITD mice need collaborating mutations to undergo leukemic transformation. These findings suggest that miR-155 inhibitors may warrant clinical consideration as therapeutics in FLT3-ITD+ AML.

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Furosemide Conserves K in Mice on a Low Na High K Diet by Inhibiting BK-mediated K Secretion in the Distal Nephron

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Understanding the effect of a cardio- and reno-protective low Na, high K diet (LNaHK) on renal K handling is crucial to choosing diuretics and anti-hypertensive agents for patients on such diets. We previously showed that furosemide, a K-wasting diuretic that inhibits the Na-K-Cl-cotransporter-2 (NKCC2) in the thick ascending limb (TAL), became a K-conserving diuretic in mice on LNaHK by inhibiting the net K secretion in TAL. Because furosemide acidifies urine by increasing acid secretion and that distal K secretion is affected by urine pH, we hypothesize that furosemide treatment also reduces distal K secretion by inhibiting the Ca-activated large conductance K channel (BK). Wild-type (WT) and BK-β4 (an accessory subunit that protects BK-α, the pore forming subunit, from lysosomal degradation) knockout mice (BK-β4 KO) were kept on LNaHK (0.01% Na, 5% K) for 7 days. After intraperitoneal (IP) injections of vehicle, furosemide (15 mg/kg), amiloride (inhibitor of ENaC-mediated driving force for distal K secretion; 5 mg/kg), or amiloride + furosemide, they were placed into metabolic cages to collect urine for 12 hours. Another group of WT were kept on LNaHK for 7 days and placed into metabolic cages to collect urine for 24 hours with access to either regular water or alkaline furosemide water (15 mg/kg/day, pH 8.8). The mice were then sacrificed and the [K] and pH were measured from blood and urine samples. Fluorescence immunohistochemistry

(FIHC) was performed on paraffin-embedded kidney sections. In WT, IP furosemide group exhibited a lower urine pH and lower urinary K clearance than the vehicle group. In BK-β4 KO, IP furosemide group exhibited a lower urine pH but a similar urinary K clearance compared to the vehicle group. IP Amiloride + furosemide group showed lower urinary K clearance than the amiloride group in both WT and BK-β4 KO. FIHC showed that BK-α was localized in the apical membrane of connecting tubules (CNT) in WT vehicle group. However, BK-α was localized in the cytoplasm of CNT in WT IP furosemide group and both groups of BK-β4 KO. Urine pH and urinary K clearance were not different between WT on LNaHK with regular water and alkaline furosemide water. These results suggest that in mice on LNaHK, in addition to suppressing the net K secretion in TAL, furosemide inhibits BK-αβ4-mediated K secretion in the distal nephron by acidifying the urine.

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Large-scale Network Functional Dysconnectivity Predicts Transition to Psychosis in Persons at Risk for Psychosis: Findings from the Longitudinal Youth-at-Risk Study

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Identification of biomarkers that predicting the clinical outcomes in persons at risk for psychosis could optimize management and intervention strategies. Psychosis has been characterized as a disorder of brain dysconnectivity. While abnormalities involving multiple neural networks have been found in individuals with an At-Risk Mental State (ARMS) for psychosis, few studies examined if the disruptions in neural networks selectively target ARMS individuals who later develop psychosis. This study attempted to identify patterns of large-scale functional brain networks dysconnectivity in ARMS individuals that predict transition to psychosis, using task-free functional magnetic resonance imaging (fMRI). Given the multidimensional characteristics of psychotic symptoms, it is important to answer these questions from a connectome-wide perspective. Therefore, we aim to examine both the interactions between functionally differentiated neural networks and the system-level network architecture in ARMS participants. **Methods:** We conducted a prospective, case-controlled, longitudinal 4-year follow-up study in Singapore. ARMS subjects were recruited from a tertiary psychiatric hospital and scanned at an academic center. We acquired the baseline task-free fMRI data from ARMS subjects who transitioned to psychosis in 4 years (ARMS-T, n=12), ARMS who did not (ARMS-NT, N=76) and healthy controls (HC, N=48). Pairwise comparisons were conducted among the three groups in whole-brain functional connectivity strengths, as well as network topology metrics: nodal efficiency and clustering coefficients. To understand the impact of FC disruptions on the system-level network architecture, we then examined the changes in brain network modularity in the two ARMS groups. Network dysconnectivity in ARMS-T was also correlated with their baseline symptom severity scores. **Results:** In comparison to ARMS-NT and HC, ARMS-T showed marked reductions in baseline functional connectivity strengths, efficiency and clustering coefficients involving widespread brain regions involving the insula, limbic system, central and peripheral visual cortex, posterior parietal cortex and somatosensory and motor cortex (P1.00). No differences were found between ARMS-NT and HC. Altered network organization was observed in the ARMS-T group, characterized by loss of network segregations and disruptions of network communities. FC disruptions in the ARMS-T group were associated with general and negative scores of Positive and Negative Syndrome Scale. **Conclusion and relevance:** Marked baseline functional dysconnectivity was found in the ARMS subgroup that subsequently developed full-blown psychosis, whereas functional connectivity in the rest of the ARMS individuals did not differ from the HC group. These findings indicate the potential of using features from the large-scale brain networks to establish clinically useful biomarkers for prognosticating outcomes in ARMS.

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Ablation of *Galc* in Schwann Cells is Sufficient to Cause Peripheral Demyelination in a Mouse Model of Globoid Cell Leukodystrophy

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Globoid Cell Leukodystrophy (GLD) is a lysosome storage disease (LSD) characterized by progressive demyelination in the central and peripheral nervous systems (CNS and PNS). GLD affects infants and progresses quickly, ultimately leading to death within two years of life. GLD is caused by mutations in GALC, leading to a loss-of-function in the lysosomal enzyme galactosylceramidase (GALC), causing toxicity to myelinating glia in the CNS (oligodendrocytes) and PNS (Schwann cells, SCs). The only treatment for GLD is Hematopoietic Stem Cell Transplantation (HSCT), a common therapy for many LSDs, which is thought to work by a process called cross-correction. Unfortunately, HSCT has limited long-term efficacy in both mice and humans for unknown reasons. One intriguing, but untested, hypothesis is that the PNS is not capable of recovery by HSCT, leading to paralysis, autonomic dysfunction and "sudden death". Similarly, recent data suggests that SCs, neurons and macrophages, each play important roles in the progression of GLD, but the autonomous role and interaction among these cells has not been delineated. To investigate these hypotheses, we have generated a *Galc* conditional knockout mouse (cKO) using the *Cre-loxP* strategy. Surprisingly, we found that the SC specific *Galc* cKO mouse has a strong demyelinating neuropathic phenotype, despite endogenous *Galc* expression in the remaining cell types. This finding contradicts our understanding of cross correction, but corroborates the clinical data from HSCT-treated GLD patients. Despite gross demyelination, SC *Galc* cKO mice showed only moderate decreases in weight loss and motor function when compared to the *Galc* KO mice, and had no mortality in 6-month survival curves. Interestingly, Sciatic nerves from SC *Galc* cKO mice were morphologically different from *Galc* KO mice. We hypothesize that that these differences are caused from a role for *Galc* in cells other than SCs, specifically macrophages and neurons. We have begun to characterize the role of these cell types in the SC *Galc* cKO mice, as well as in motor neuron *Galc* cKO mice, macrophage *Galc* cKO mice and compound PNS *Galc* cKO mice. This work will help us to better understand the pathophysiology of GLD in the PNS, which should have direct implication for further studies in the CNS. In addition, findings from this study will clarify disease mechanisms of GLD and the limitations of HSCT, which will allow us to develop better therapies for GLD in the future. Our data provide information on the interplay between glia and neurons in neurodegeneration and neuroinflammation, a central emerging question in neurology.

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Peripheral Nerve Grafts to the Brain of Patients with Parkinson's Disease: Microscopic, Biochemical, and Immunohistochemical Characterization

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Currently two clinical trials (NCT01833364 and NCT02369003) are underway which feature the implantation of a peripheral nerve autograft to the brain (targeted either to the Substantia Nigra or the Nucleus Basalis of Meynert) in combination with Deep Brain Stimulation (DBS) for the treatment of patients with Parkinson's disease. This nerve tissue is harvested from the sural nerve, a cutaneous sensory nerve located in the lateral ankle, of patients undergoing DBS surgery. Two tissue samples per patient are collected for study (one during the Stage I surgery, another during the Stage II surgery 5-14 days later) in addition to the tissue used for the graft. As of 12/13/16, 36 patients have received a graft. The character of the peripheral nerve tissue used in these clinical trials has yet to be described. This study examines several aspects of the peripheral nerve tissue; including microscopic appearance, levels of neurotrophic factors, morphology of Schwann Cells, and presence of macrophages. Techniques used include H&E and MCOLL histological staining, immunohistochemistry, and ELISA. These results are supplemented by immunohistochemical analysis of the brain of non-human primates that have undergone an analogous procedure. The results of this model show growth of tyrosine hydroxylase-containing nerve fibers, which are a marker of dopamine-producing neurons, into the area of the peripheral nerve graft. In addition, results in this model show the presence of S100beta-containing cells as well as GFAP-containing cells within and surrounding the graft, which is a marker of peripheral nerve regeneration. These findings suggest that the nerve graft in human patients may also display a regenerative phenotype which has the potential to alter the course of neurodegeneration in the brain.

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Circuit-specific Genomic and Functional Dissection of Male and Female Resilience to Social Stress

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Depressive syndromes are a major cause of morbidity, affecting nearly 300 million globally, and often arise in response to life stress. Aside from its obvious social and financial burden, a striking characteristic of depression is that it affects women nearly twice as often as men. The impact of depression and the disparity in numbers of affected men and women have been recognized for some time, but the molecular underpinnings of the disease remain unknown. This knowledge gap is critical, as treatments remain ineffective in a large number of patients and no sex-specific therapies are known. Neurons in the hippocampus, particularly those that project to the nucleus accumbens, are mediators of stress responses, but little is known of the regulation of this circuit at the level of cell function or gene expression. Here, I use a novel witness chronic social defeat stress (CSDS) mouse model that is amenable to both sexes to investigate the role of the transcription factor ΔFosB in the regulation of ventral hippocampal-nucleus accumbens (vHPC-NAc) projection cells in susceptibility or resilience to CSDS. This circuit has gained recent attention in mood disorder research as it has been shown that reduced activity in these neurons promotes resilience to CSDS. Additionally, our group has shown that the transcription factor ΔFosB is required for hippocampal learning, and its expression is induced in the vHPC by stress or antidepressant treatment. Taken together with our knowledge of its role in resilience in other brain regions, this makes ΔFosB an exciting prospective target for the regulation of vHPC-NAc neuronal function in response to stress. Here, I show that general inhibition of ΔFosB function throughout the vHPC (but not dHPC) promotes susceptibility to subchronic stress and that overexpression of ΔFosB in vHPC reduces cell excitability. With these findings, I hypothesize that stress-induced ΔFosB in vHPC-NAc neurons mediates changes in the function of these neurons and regulates gene expression to promote resilience in male and female mice.

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Lateral Hypothalamic Neurotensin Neurons Engage the Mesolimbic Dopamine System to Promote Weight Loss Behaviors

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Mesolimbic dopamine (DA) neurons in the ventral tegmental area (VTA) modify feeding and locomotor behaviors that impact body weight, and may be useful targets to treat or prevent obesity. We sought to understand how the neuropeptide neurotensin (Nts) engages the mesolimbic DA system and whether it may be useful to support weight loss. Previous work demonstrates that direct application of Nts to the VTA activates DA neurons expressing the Gq-coupled neurotensin receptor 1 (NtsR1), suppresses feeding and promotes physical activity, however the endogenous source of Nts input to the VTA remains unclear. To investigate this, we injected a retrograde tracer into the VTA of *Nts^{Cre};GFP* reporter mice and found that Nts neurons in the lateral hypothalamic area (LHA) provide significant Nts input to the VTA. We therefore hypothesized that activation of the LHA Nts→ VTA DA circuit promotes weight loss behaviors, and that Nts action via NtsR1 is crucial for this effect. To interrogate this, we used DREADDs to specifically activate LHA Nts neurons in wild-type (WT) mice and mice that lack NtsR1 (NtsR1KO mice). Activation of LHA Nts neurons increased locomotor activity and energy expenditure in WT and NtsR1KO mice, which was blunted by a DA receptor 1 (DR1) antagonist, confirming the requirement of DA signaling for promoting physical activity via this circuit. However, pretreatment with an NtsR1 antagonist did not blunt physical activity or energy expenditure, indicating that LHA Nts neurons induce locomotor behavior via an NtsR1-independent mechanism. Interestingly, activation of LHA Nts neurons in WT mice promotes energy expenditure without a compensatory increase in feeding which may promote weight loss over time. Indeed, repeated activation of the LHA Nts→ VTA DA circuit in WT mice induced a net energy deficit and weight loss in these mice. By contrast, activation of LHA Nts neurons in NtsR1KO mice or in WT mice pre-treated with an NtsR1 antagonist increased food intake, suggesting that Nts action via NtsR1 is required to restrain compensatory feeding. Together, these data reveal that activation of the LHA Nts → VTA DA circuit promotes weight loss behaviors via Nts-dependent and Nts-independent mechanisms. We therefore tested the translational potential of this circuit for treating obesity by activating LHA Nts neurons in diet-induced obese mice. Contrary to our hypothesis, repeated activation of LHA Nts neurons did not induce weight loss behaviors in severely obese mice, indicating that the LHA Nts → VTA DA circuit becomes dysfunctional in late-stage obesity. Collectively, these data suggest that therapeutic enhancement of LHA Nts signaling may be useful to support behaviors that prevent the development of obesity.

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Disturbed Flow Stabilizes Hypoxia Inducible Factor-1alpha Dependent Metabolic Reprogramming in Endothelial Cells and Modulates Endothelial Inflammation

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Mechanical stimuli regulate major cellular functions that are critical to health and disease in humans. This is especially important in the vasculature where, in branching regions of arteries, local disturbed flow activates endothelial cells (ECs) resulting in vascular leak and inflammation. This ultimately leads to atherosclerosis, the major cause of coronary artery disease. Increasing evidence suggests that metabolic reprogramming may play a role in EC activation; however, how flow phenotypes alter EC metabolism is not completely understood. In the current study, whole genome RNA-sequencing and pathway analyses identified that glycolytic metabolism was significantly activated in ECs cultured under disturbed flow. Bioenergetics measurements confirmed that disturbed flow significantly increased glycolysis and simultaneously reduced respiratory capacity in ECs. Transcription factor hypoxia inducible factor-1α (HIF-1α) was required for both activation of glycolysis and inhibition of oxidative phosphorylation in ECs under disturbed flow. Mechanistically, disturbed flow stabilized endothelial HIF-1α, leading to increased expression of glycolytic genes (major glucose transporter SLC2A1, hexokinase-2 (HK2)) and enhanced pyruvate dehydrogenase kinase-1 (PDK-1), limiting pyruvate availability for mitochondrial use. Increased NAD(P)H oxidase 4 (NOX4) expression and consequent elevated reactive oxygen species (ROS) production stabilized HIF-1α in ECs under disturbed flow. Furthermore, swine aortic arch endothelia in areas of disturbed flow also exhibited higher NOX4, ROS, HIF-1α, SLC2A1, HK2, and PDK1 expression, suggesting a regulatory role for hemodynamics in acting as a metabolic switch *in vivo*. Finally, inhibition of glycolytic genes reduced endothelial inflammation. These new molecular insights collectively highlight the significance of mechanical regulation of cellular metabolism and downstream inflammation, particularly in endothelium constantly exposed to diverse hemodynamic forces.

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Investigating the Roles of p63 Isoforms in Lung Progenitor Cells and Lung Tumorigenesis

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Non-small cell lung cancer has a poor prognosis and lack of effective treatments, identifying a need to better understand its molecular basis and pathogenesis. Cancer may originate from transformed adult stem cells, due to similarities in signaling pathways and self-renewal abilities. The transcription factor p63 is a master regulator of epithelial development and differentiation and has a role in stem cell maintenance of multiple epithelial tissues. p63's role has been difficult to characterize as it was discovered that it has two isoforms with distinct functions, TAp63 and ΔNp63. Our lab has established that p63 isoforms regulate distinct stem cell compartments in the skin. The p63 isoforms also function as tumor suppressors. Our lab has shown that TAp63 suppresses metastatic lung adenocarcinomas. Importantly, we have also identified novel ways of therapeutically targeting cancers through manipulation of ΔNp63. The transcription factor p63 is known to be expressed in the basal stem cells of the lung however, the physiological roles of individual p63 isoforms, TAp63 and ΔNp63, have not been characterized. Through the characterization of lung progenitor cells regulated by p63 isoforms, our project aims to provide important insights about lung biology and the cell of origin in different lung cancer subtypes. To understand the roles of individual p63 isoforms, we utilized *TAp63* and *ΔNp63* conditional knock out mice generated by our lab. To specifically target the trachea and lungs for recombination, we used an intubation technique that involves administering adenoviral Cre Recombinase directly into the mouse trachea to induce recombination in the trachea and lungs, with characterization at time points to assess the effect on different stem cell populations by staining with lung stem cell markers. We have found a role for the ΔNp63 isoform in the maintenance of tracheal basal stem cells and direction of lung stem cell fate. Mice with knockout of ΔNp63 exhibited initial hyperproliferation of the Krt5+ basal cell population in the tracheal epithelium but demonstrated epithelial hypoplasia of the trachea with loss of basal cells at a later time point, suggesting *ΔNp63* plays a role in maintenance of this progenitor cell population. By isolating the basal cells *in vitro*, we observed that loss of ΔNp63led to impairment of basal cell sphere formation and terminal differentiation in 3D culture. Through a combination of RNA-seq and ChIP-seq analysis of the tracheal basal cells, we have identified a transcriptional network of genes regulated by ΔNp63, including epithelial development, proliferation, epithelial to mesenchymal transition and metabolism, that were also differentially altered in the TCGA lung squamous cell carcinoma cohort. Our research provides new insights to the under-characterized p63 gene in lung cancer while investigating a novel regulatory process for progenitor cells of the lung.

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Costimulated, Immunotherapeutic CD8 T Cells Synergistically Produce IFNγ When Provided a Signal-3 Combination of IL-2 and IL-36

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It has been well established that priming CD8 T cells with TNFR superfamily members 4-1BB (CD134) and OX40 (CD134) (termed dual costimulation; DCo) boosts their capacity to mount effector T cell responses. More recently, it was discovered that on days 4-5 after *in vivo* priming with DCo, CD8 T cells exhibit a transient capacity to undergo aerobic glycolysis. This transient metabolic phenotype furthermore tracked with a capacity to also produce robust signal 3 -cytokine mediated release of IFNγ. When cultured with IL-2 and the IL-1 family member IL-36, primed CD8 T cells release a synergistic amount of IFNγ, as early as 2 h after cytokine stimulation. This synergistic IFNγ production could furthermore be elicited from the draining lymph nodes and tumor homogenates of DCo-treated mice bearing B16 melanoma tumors. To explore the mechanism behind this robust synergy in IFNγ production, RNAseq analysis was performed on CD8 T cells stimulated with media alone, IL-2, IL-36, or the combination IL-2 and IL-36, on days 4-5 after *in vivo* priming. From our analyses, we identified transcriptional factor pathways unique to the IL-2 and IL-36 combination that lay upstream of IFNγ. Provided the established antitumor effects of IFNγ, the synergy of its production when IL-2 and IL-36 are simultaneously offered is a response that would possibly be beneficial to exploit in the immunotherapy of cancer.

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A HIF-2a-long Noncoding RNA Epigenetic Axis Modulates Histone Methylation and Mitochondrial Metabolism in Hypoxia: Implications for the Pathogenesis of Pulmonary Hypertension

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Long noncoding RNAs (lncRNAs) are expressed extensively in the mammalian vasculature, yet their potential involvement in regulating the hypoxic response and pathogenesis of pulmonary hypertension (PH) is unknown. Specific lncRNAs control the dysregulated metabolic and proproliferative states of endothelial cells induced by hypoxia and are central to the development of PH. **Methods:** Eight-week-old mice (C57BL/6) were injected with SU5416 (20 mg/kg/dose; VEGFR antagonist), followed by exposure to normobaric hypoxia (10% O_2) for 2 weeks to induce PH. Transcriptomic profiles were generated by next generation RNA sequencing (Illumina), and differential expression analysis was performed. siRNA-mediated gene silencing and lentivirus-mediated overexpression were performed to control lncRNA expression in human pulmonary artery endothelial cells (PAECs). Chromatin immunoprecipitation coupled with quantitative PCR (ChIP-qPCR) was performed to analyze enrichment of HIF binding motifs at the KMT2E-AS1 promoter and methylated histone at the HIF-2α promoter. Cell apoptosis was measured by caspase-3/9 activity assay and proliferation capability was evaluated by BrdU incorporation. Mitochondrial oxygen consumption and glycolytic

flux were measured (Seahorse assay). **Results:** RNA sequencing revealed increased expression of the lncRNA 5031425E22RIK in PH mouse lung, which was further confirmed by its increased expression in cultured mouse PAECs exposed to hypoxia. Its human ortholog KMT2E-AS1 is an antisense gene of a hypoxia-dependent histone lysine(K)-specific methyltransferase, a gene important in regulating chromatin remodeling and transcriptional activation. KMT2E-AS1 also exhibited elevated expression in hypoxic human PAECs and in lung tissues of PH patients. Knockdown of a master transcription factor of hypoxia, HIF-2α, but not HIF-1α, prevented upregulation of KMT2E-AS1 in hypoxia, whereas overexpression of a constitutively active HIF-2α promoted its expression in normoxia. Luciferase assay and ChIP-qPCR verified HIF-2α-induced enrichment of HIF binding motifs in the KMT2E-AS1 promoter. More importantly, KMT2E-AS1 directly interacted with and up-regulated the expression of KMT2E and other KMT2 family members in hypoxia and controlled histone H3 lysine 4 trimethylation (H3K4Me3). More importantly, KMT2E-AS1 knockdown reduced H3K4Me3 enrichment at the HIF-2α promoter site, and increased expression of prolyl hydroxylase2 and UBE2D, two factors in the hydroxylase-ubiquitin pathway controlling HIF inactivation. RNA sequencing further revealed that KMT2E-AS1 knockdown inhibited hypoxia-activated HIF signaling with downstream reprogramming of metabolic genes controlling oxidative phosphorylation and glycolysis. Correspondingly, as demonstrated by gain- and loss-of-function analysis of this lncRNA and consistent with its control of HIF, KMT2E-AS1 was found to be both necessary and sufficient to promote glycolytic flux, repress mitochondrial oxidative metabolism, and thus induce a hyperproliferative state in hypoxic PAECs. **Conclusions:** In hypoxic PAECs, mouse lncRNA 5031425E22RIK and its human ortholog KMT2E-AS1 engage in an epigenetic regulatory feedback loop with HIF-2a to control histone methylation, leading to metabolic reprogramming and a proproliferative endothelial phenotype. These biological functions may play critical roles in the pathogenesis of pulmonary hypertension.

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The Professional Decision-Making in Medicine Measure: Development and Preliminary Results

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In this presentation, I report on the development and preliminary results of the Professional Decision-Making in Medicine (PDM) measure, a vignette-based test that investigates decision-making strategies used by medical students when faced with challenging situations in the context of clinical practice. The procedure to develop the PDM was extensive to ensure optimal realism and clarity: (1) identification of topics areas for medical professionalism by cross-referencing key literature in the field, (2) physician interviews to identify and understand professional challenges in clinical practice to inform content for the measure, (3) interdisciplinary team meetings to thoroughly inform the writing process throughout development, and (4) cognitive interviews with a diverse group of physicians to identify problems with the PDM items or response options that required revision or clarification. From the interviews and writing process, the final version of the PDM contained five case scenarios followed by three to four vignette-based items, for a total of 16 items. Each item has six possible response options, three "less" professional and three "more professional" options, of which the respondent must choose two correct ("more" professional) options to receive a point for the item. "More" professional indicates the use of compensatory strategies in the decision-making process. The PDM was written at an eighth grade reading level, and the scenarios covered a range of clinical settings and specialties. To gather preliminary data, the research team administered the PDM through an online surveying tool with a battery of validity measures to a group of fourth-year medical students (N = 151) who were diverse in terms of age, race, nationality, native language, and medical specialty chosen. The data show mixed results. The PDM demonstrated poor reliability (KR alpha = .44) even though the test's design was based on another measure with good reliability (alpha = .84). The mean PDM score was 12.01 (SD = 2.06) out of 16, with about one quarter of respondents scoring low (10 or less). PDM scores significantly correlated negatively with foreign nationality and non-English native language and significantly correlated positively with race. Other correlations could become significant with a larger sample size. These preliminary results demonstrate further research into the possible roles of culture and medical professionalism training is warranted. Additional research and instrument refining could demonstrate the potential usefulness of the PDM as an educational outcome assessment tool and a research instrument for empirical studies on medical professionalism.

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Myeloid Specific H-ferritin Mediates Sepsis Induced Inflammation and Organ Injury

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Sepsis is a severe clinical syndrome that is characterized by profound and dysregulated inflammatory response to infection resulting in end-organ dysfunction distant from the primary site of infection. Despite fundamental findings that have expanded our understanding into the mechanisms that instigate and propagate sepsis and its deleterious effects on various organs including kidney, novel therapeutic agents and modalities have remained elusive. In fact, sepsis remains a leading cause of mortality and acute kidney injury in patients admitted to the intensive care unit. We previously demonstrated that macrophage polarization depends on expression of ferritin heavy chain (FtH) and such expression plays a key role to regulate the cross-talk between macrophages and renal epithelial cells during kidney injury and repair. This led to our hypothesis that macrophage specific FtH may be involved in development and consequences of sepsis. Using transgenic mice with conditional deletion of FtH in myeloid cells (FtH $LysM^{-/-}$), we induced sepsis by a well characterized cecal ligation and puncture method. Our results demonstrate that myeloid FtH deficiency is associated with hyporesponsiveness to sepsis. We show that specific deletion of FtH in myeloid cells led to ~90% improved survival when compared to FtH $LysM^{+/+}$ littermates. Furthermore, renal function supported by serum creatinine was significantly more preserved in the FtH $LysM^{-/-}$ mice. In addition, we found decreased level of several pro-inflammatory cytokine expression in major organs, including the kidney. Our mechanistic studies show that myeloid FtH deletion causes derangements in multiple pathways that are crucial in innate immunity and inflammation including NF-κB, hypoxia inducible factor and immunoresponsive gene-1. Overall, our results for the first time signify the paramount importance of myeloid system iron metabolism in sepsis mediated organ injury and identify the central role of FtH

in this context. As such, we propose a novel target to mitigate sepsis mediated inflammation and consequent organ injury that is urgently needed given the unacceptable rate of mortality and morbidity related to this devastating clinical condition.

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The Source of Succinate in Myocardial Ischemia

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Maladaptive metabolic events are responsible for the oxidative damage seen in cardiac ischemia-reperfusion (IR) injury, the underlying pathology of acute myocardial infarction. The metabolite succinate is known to accumulate in ischemic myocardium, and is rapidly consumed at reperfusion, driving the generation of reactive oxygen species. Despite this detrimental role at reperfusion, ischemic succinate accumulation is evolutionarily conserved across diverse tissues and species, suggesting that it serves a beneficial role in ischemia. Elucidating the pathway of succinate accumulation can provide insights into ischemic metabolism for the development of novel therapeutics. Herein, we investigate the hypothesis that ischemic succinate accumulation results from a functional electron transport chain (ETC) of Complexes (Cx) I and II (CxI-II ETC). **Methods and results:** Isolated mouse heart mitochondria were respired in a Clark electrode until hypoxic (in vitro spectrophotometrically. Metabolomics analysis of perfused hearts subjected to ischemia revealed increased abundance of branched-chain amino acid degradation intermediates, a catabolic pathway that lies upstream of succinate. Finally, in perfused hearts subjected to IR injury (25 min. ischemia, 60 min. reperfusion), delivery of the CxII inhibitor Atpenin A5 (AA5) at reperfusion, but not prior to ischemia, improved cardiac functional recovery and reduced infarct size. **Conclusions:** Herein, we show that while hypoxic mitochondria are capable of generating succinate via the CxI-II ETC, this was not the mechanism for ischemic succinate accumulation in intact hearts. Additionally, since succinate generation was not sensitive to CxI, CxII or Krebs cycle inhibitors, the source of succinate in ischemic hearts likely does not require the ETC. A preliminary metabolomics approach implicated branched-chain amino acid degradation as a putative pathway for ischemic succinate generation. This hypothesis will be the subject of further investigation. Finally, AA5 was cardioprotective when delivered at reperfusion, but not pre-ischemia, highlighting the importance of identifying drug delivery windows in IR injury. CxII inhibition at reperfusion via AA5 analogs might represent a novel pharmacologic strategy of acute myocardial infarction treatment.

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Resolution of Hyperoxia Induced Acute Lung Injury is Dependent Upon TLR4

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Animal models have demonstrated that hyperoxia leads to elevated levels of oxidants that damage both pulmonary epithelial and endothelial cells, thereby causing increased pulmonary capillary permeability, inflammation, and eventual respiratory demise – consistent with clinical acute lung injury (ALI). However, very little is known about mechanisms of repair after ALI. During the repair phase, alveolar fluid is reabsorbed, inflammatory response attenuated, and collagen fibers facilitate cellular migration. Our previous study showed that toll-like receptor 4 (TLR4) confers protection against hyperoxia-induced ALI and endothelial cell injury. We sought to elucidate the role of TLR4 in the recovery phase of hyperoxia-induced lung injury (HALI). **Methods:** Wild type (Wt) and $TLR4^{-/-}$ mice were exposed to 100% oxygen. Lung injury was assessed after 48 hours of exposure and during the recovery phases (1, 2, 3, 6 and 9 days). Bronchoalveolar lavage (BAL) fluid and lung tissue were obtained and analyzed. **Results:** Hyperoxia caused a significant increase in BAL total cell counts and lung cellular infiltrates, which consisted of macrophages, lymphocytes, and neutrophils. As expected, $TLR4^{-/-}$ mice were significantly more susceptible to hyperoxia than WT mice. However, during the early lung repair phase (post-hyperoxia 1~3 days), lung inflammation and vascular leak decreased to room air levels in WT mice but not in $TLR4^{-/-}$ mice, as assessed by protein content, LDH, and H_2O_2 production in BAL. Sirius Red staining showed the deposition of collagen fibers around the alveoli in the early recovery phase and disappearance of the collagen in the latter recovery phase. $TLR4^{-/-}$ mice demonstrated significantly delayed repair compared with WT mice. We identified significantly lower levels of mitochondrial protein, uncoupling protein 2 (UCP2), in $TLR4^{-/-}$ mice, suggesting a potential role for UCP2-mediated mitochondrial processes in post-hyperoxia recovery. We have also initiated extensive immunologic profiling of cell populations in lungs, BAL and bone marrow using multi-channel flow cytometry and CyTOF (cytometry-based time-of-flight) technology, which identified unique cell populations and kinetics of inflammation. **Conclusions:** We identified a role for TLR4-mediated lung repair after hyperoxia-induced lung injury. Repair after hyperoxia was associated with resolution of alveolar protein leak, cellular inflammation, oxidant production and collagen accumulation. TLR4-deficiency was associated with impaired resolution and decreased expression of UCP2 in lungs. Future studies will investigate the specific roles of UCP2, as well as mitochondrial bioenergetics, in the resolution phase of hyperoxia-induced lung injury, validate our single cell-identification approaches using CyTOF and initiate real-time intravital imaging in the recovering lungs.

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Mouth and Voice: A Relationship Between Visual and Auditory Stimulus Selectivity in the Human Superior Temporal Sulcus

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Speech perception is a multisensory process that uses both visual information from the talker's face and auditory information from the talker's voice. The superior temporal sulcus (STS) is a key brain locus for multisensory integration but little is known about its neuroanatomical organization. Previous studies have shown that subregions of the STS respond to visually-presented mouth movements, visually-presented eye movements, and auditory stimuli. Because we usually see visual mouth movements at the same time as we hear vocal speech, we hypothesized that regions of the STS with a preference for visual mouth movements should respond strongly to auditory stimuli. To test this hypothesis, we used blood oxygenation level dependent (BOLD) functional magnetic resonance imaging (fMRI) to scan twenty healthy subjects as they watched silent videos of moving faces or listened to auditory clips of continuous speech, vocal sounds, and non-vocal sounds. In each subject, we identified STS voxels that showed a preference for visual mouth movements. These voxels showed a greater response to auditory speech than eye-movement preferring voxels (1.4 ± 0.2% vs. 0 ± 0.1%, $p=3.8x10^{-10}$) with a positive correlation between mouth-preference and auditory response (m=0.22, $r^2=0.77$, p=0.009). Next, we examined whether the auditory response was selective for vocal stimuli. Mouth-preferring voxels responded more to vocal than non-vocal stimuli (1.2 ± 0.1% vs. 0.5 ± 0.1%, $p=5.9x10^{-6}$) with a correlation between mouth-preference and vocal-preference (m=0.18, $r^2=0.96$, $p=1.3x10^{-4}$). The converse was also true: regions that showed a significant response to speech responded more strongly to moving mouths than moving eyes (0.37 ± 0.04% vs. 0.17 ± 0.03%, $p=1.5x10^{-8}$). Our study demonstrates that subregions of the STS respond strongly to both visually-presented mouth movements and auditory speech, suggesting that these stimulus features are coded together in small populations of STS neurons. By showing a link between visual and auditory selectivity in the human pSTS, our results help to reveal the organizing principles underlying neural computations that subserve one of our most important abilities: the ability to understand speech.

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Rotavirus Infection Induces LGR5 Intestinal Stem Cell Population via WNT Pathway Stimulation

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The study of stem cell responses to injury has broad implications for understanding divergent roles of stem cell populations. Traditional methods to study the intestinal stem cells (ISCs) under injury use γ-irradiation to target proliferating stem cells. Previous work showed LGR5-labelled ISCs actively proliferate to maintain homeostasis and are highly susceptible to γ-irradiation injury. BMI1-labelled ISCs are normally quiescent but can activate to respond under injury. Here, we use rotavirus (RV) infection *in vivo* and in the Human Intestinal Enteroids (HIEs) as a novel injury model to examine ISC populations and their induction under insult. **Methods**. Transgenic mouse models, $Lgr5^{CreER\text{-}GFP}$ and $Bmi1^{CreER};R26^{mTmG}$, were used to examine LGR5- and BMI1-labelled ISC populations. HIEs were used to model RV infection in a human, epithelial system. We examined WNT pathway target genes using qRT-PCR and canonical β-catenin activity via TCF/LEF luciferase reporter assay. **Results**. In contrast to γ-irradiation, RV infection targets differentiated cells, thus retaining an intact ISC compartment with both LGR5- and BMI1-labelled populations. When compared with mock-infected animals, mice infected with RV show a lengthened and more proliferative ISC compartment. Proliferative marker PCNA is significantly increased at both the mRNA and protein levels after RV infection. Additionally, proliferating cells labeled by EdU migrate faster out of the crypt compartment following infection. To compare ISC involvement, we infected $Lgr5^{CreER\text{-}GFP}$ and $Bmi1^{CreER};R26^{mTmG}$ mice. Both Lgr5 transcripts and cell number are significantly increased after infection compared to mock animals. Although Bmi1 transcripts are increased following infection, BMI1 cell numbers remain constant. Similar findings are seen in RV-infected HIEs, suggesting that actively-cycling LGR5 cells are the bona fide ISC responder under RV damage. To examine how ISCs are activated following infection, we screened for gene expression involved in the WNT pathway (*Axin2, Ccnd1, Cd44, EphB2, Myc, and Sox9*). We found that all screened genes are upregulated following infection in both mice and HIEs. Furthermore, conditioned media from RV-infected HIEs stimulated canonical β-catenin activity in a TCF/LEF luciferase reporter assay and, when used to culture naïve, un-infected HIEs, can further stimulate the WNT signaling pathway. **Conclusions**. In contrast to γ-irradiation, RV infection of the differentiated cell types induces the LGR5 ISCs and *not* the BMI1 cells. This induction depends on canonical WNT signaling pathway activation, which leads to proliferation and repair of the intestinal epithelium. Our data suggest that, when LGR5 population is intact, it remains the primary source of epithelial restitution and does not rely on BMI1 cells, irrespective of insult.

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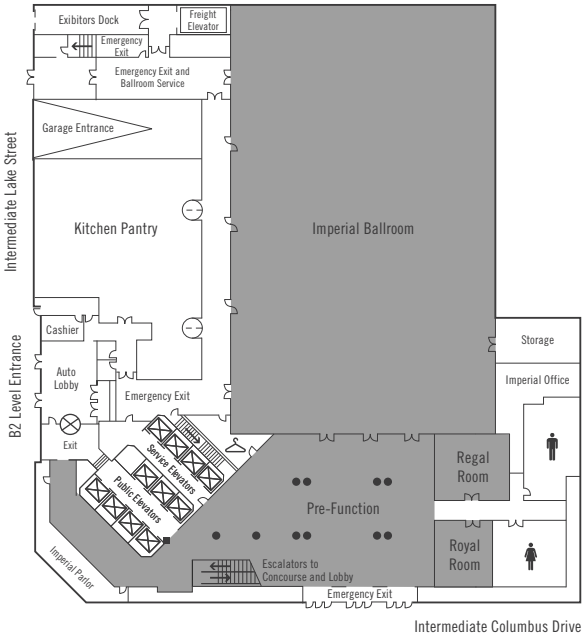
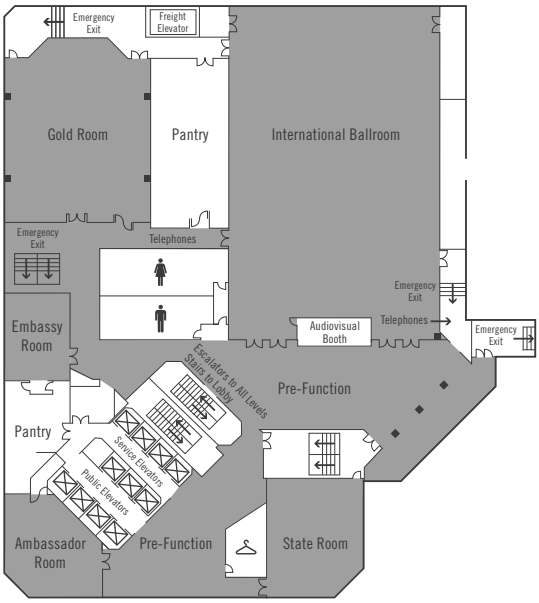
Notes

Hotel Floor Plans

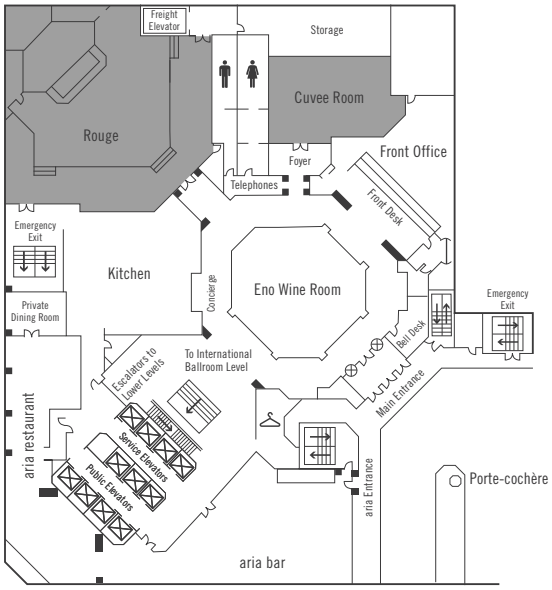
Imperial Ballroom Level (B2 level)



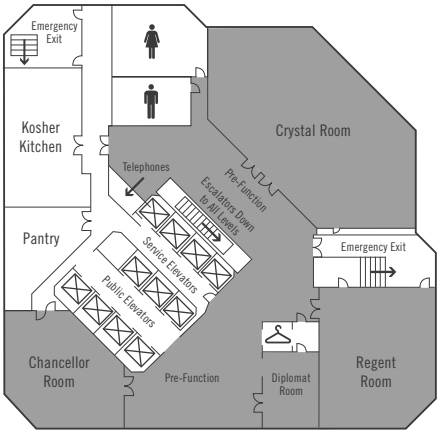
International Ballroom Level (2nd level)



Lobby Level (1st level)



Meeting Room Level (3rd level)



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