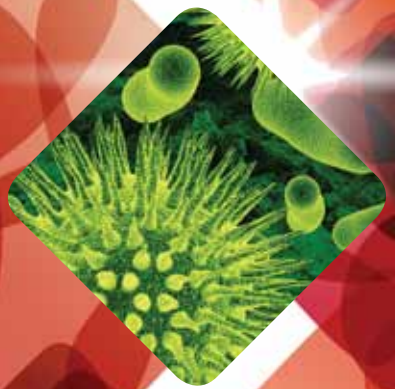
Meeting Program and Abstracts

ASCI / AAP Joint Meeting 2012

April 27 – 29, 2012

The Fairmont Chicago
Chicago, Illinois



American Society for Clinical Investigation 1908

APSA
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Special Events at the 2012 ASCI/AAP Joint Meeting

Friday April 27

ASCI Welcome Reception

6 – 7 p.m.
Imperial Ballroom Foyer (Level B2)

OPEN TO ALL MEETING ATTENDEES

The ASCI Welcome Reception provides a relaxed social environment for attendees of the 2012 ASCI/AAP Joint Meeting to begin their weekend. Presentations for the Poster Session will be set and ready for viewing.

ASCI Dinner and Induction Ceremony

7 – 9 p.m.
Moulin Rouge, 1st Floor

TICKETED GUESTS ONLY

The ASCI welcomes its new members for 2012 at its annual dinner.

Speaker: The Honorable Bill Foster, former U.S. Representative

Saturday April 28

AAP Reception and Dinner

7 – 10 p.m.
Imperial Ballroom

TICKETED GUESTS ONLY

The AAP welcomes its new members for 2012 to the annual dinner. The reception begins at 7 p.m. in the Imperial Ballroom Foyer (Level B2), followed by dinner in the Imperial Ballroom at 8 p.m.

Connected: The Surprising Power of Our Social Networks and How They Shape Our Lives
Speaker: James H. Fowler
University of California, San Diego

Dessert Reception

10 p.m. – Midnight
Imperial Ballroom Foyer

OPEN TO ALL MEETING ATTENDEES

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General Information

Accreditation and Credit Designation

This activity has been planned and implemented in accordance with the Essential Areas and policies of the Accreditation Council for Continuing Medical Education through the joint sponsorship of the University of Chicago Pritzker School of Medicine and **The American Society for Clinical Investigation and the Association of American Physicians.** The University of Chicago Pritzker School of Medicine is accredited by the ACCME to provide continuing medical education for physicians.

The University of Chicago Pritzker School of Medicine designates this live activity for a maximum of **9.5** *AMA PRA Category 1 Credits*™. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

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

Learning Objectives

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

The 2012 ASCI/AAP Joint Meeting program will feature lectures by accomplished researchers who will discuss state-of-the-art advances in their respective fields. The program is designed to foster in-depth discussions and close interactions among the meeting participants.

Meeting Evaluation

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

Poster Session Schedule

Friday, April 27

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

Saturday, April 28

Viewing	7:00 a.m. – Noon
Presentation	Noon – 1:30 p.m.
Dismantle	1:30 p.m. – 2:00 p.m.

Presenters: you must be at your posters from Noon – 1:30 p.m. on April 28. A faculty member will visit presenters during this time to discuss your work.

Best Poster Awards

Best Poster Awards will be given in the amount of $1,000 each. Members of the ASCI, AAP, and APSA will judge posters on scientific novelty, quality and clarity of presentation. Awards will be presented on Sunday from 8:15 – 8:30 a.m.

ASCI Membership Desk

Visit the ASCI membership desk in the foyer of the International Ballroom for a list of new members and to pick up complimentary recent issues of *The Journal of Clinical Investigation*.

AAP Membership Desk

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

Registration Hours

Friday, April 27	7:00 a.m. – 7:00 p.m.
Saturday, April 28	7:00 a.m. – 5:00 p.m.
Sunday, April 29	7:00 a.m. – 10:00 a.m.

Scientific Program

Friday, April 27

Time	Event	Location
7:00 a.m. – 7:00 p.m.	Registration	International Ballroom Foyer
1:00 p.m. – 3:00 p.m.	Poster Setup	Imperial Ballroom
1:00 p.m. – 3:30 p.m.	**APSA Plenary Session** Moderator: Taylor Heald-Sargent	Moulin Rouge
1:00 p.m. – 1:30 p.m.	**APSA Keynote – Roger Lewis, MD, PhD, FACEP** *Harbor-UCLA Medical Center*	
1:30 p.m. – 2:00 p.m.	**APSA Keynote – Elizabeth Engle, MD** *Children's Hospital Boston, Harvard Medical School* **Flexible Foundations for a Medical Research Center**	
2:00 p.m. – 3:00 p.m.	**APSA Session I: Women in Medicine Panel** **Elizabeth Engle, MD,** *Harvard University* **Beverly Mitchell, MD,** *Stanford University* **Theodora Ross, MD, PhD,** *The University of Texas Southwestern Medical Center at Dallas* **Vivian G. Cheung, MD,** *Children's Hospital of Philadelphia* **Serpil Erzurum, MD,** *Cleveland Clinic*	
3:00 p.m. – 3:30 p.m.	**APSA Keynote – Frederick S. Kaplan, MD** *University of Pennsylvania* **Why Do Some People Form Two Skeletons?**	
3:30 p.m. – 6:00 p.m.	**Plenary Session I – Understanding Disease Mechanisms to Improve Human Health** Moderators: Warner C. Greene, William C. Hahn and Jennifer Kwan	International Ballroom
3:30 p.m. – 4:00 p.m.	**Jenny Ting, PhD** *University of North Carolina* **NLR Innate Immune Sensors in Diseases and Disease Models**	
4:00 p.m. – 4:30 p.m.	**Olufunmilayo Olopade, MD** *The University of Chicago* **Closing the Knowledge Disparity Gap:** **From Molecular Mechanisms to Interventions and Back**	
4:30 p.m. – 4:45 p.m.	**Presentation of Career Development Awards**	
4:45 p.m. – 5:15 p.m.	**Craig Venter, PhD** *J. Craig Venter Institute* **Synthetic Life**	
5:15 p.m. – 5:45 p.m.	**Ramesh Rao, PhD** *University of California, San Diego* **Information Technology for Disruptive Changes in Medicine**	
5:45 p.m. – 6:00 p.m.	Q&A	
6:00 p.m. – 7:00 p.m.	ASCI Welcome Reception and Poster Viewing	Imperial Ballroom and Foyer
6:00 p.m. – 9:30 p.m.	Poster Viewing	Imperial Ballroom
7:00 p.m. – 9:00 p.m.	**ASCI Annual Dinner/Introduction of New Members** Speaker: The Honorable Bill Foster, former U.S. Representative	Moulin Rouge

Scientific Program

Friday, April 27

Time	Event	Location
7:00 p.m. – 9:00 p.m.	**APSA Dinner Outing** Speaker: **Ivayla I. Geneva, MD-PhD candidate** (On Your Own) Groups will meet at the Registration Desk, Fairmont Hotel	Off site
9:00 p.m. – Midnight	**APSA Welcome Reception/APSA Presidential Address** Speaker: **Ivayla I. Geneva, MD-PhD candidate** *State University of New York - Upstate*	Off site

Saturday, April 28

7:00 a.m. – 5:00 p.m.	Registration	International Ballroom Foyer
7:00 a.m. – 8:15 a.m.	Continental Breakfast	International Ballroom Foyer
7:00 a.m. – 8:15 a.m.	**Young Investigators' Mentoring Breakfast**	Moulin Rouge
7:00 a.m. – 1:30 p.m.	Poster Viewing	Imperial Ballroom
8:15 a.m. – Noon	**Plenary Session II – Understanding Disease Mechanisms to Improve Human Health** Moderators: David A. Brenner, Elizabeth McNally and Dania Daye	International Ballroom
8:15 a.m. – 8:45 a.m.	**Michael Karin, PhD** *University of California, San Diego* **In the Footsteps of Virchow: Tumor Elicited Inflammation – Mechanisms and Outcome**	
8:45 a.m. – 9:15 a.m.	**Matthew Meyerson, MD, PhD** *Dana-Farber Cancer Institute* **Somatic Genome Alterations and Targeted Therapy in Human Epithelial Cancers**	
9:15 a.m. – 9:30 a.m.	**Oral Abstract Presentation** **Neeha Zaidi, BS** *Mount Sinai School of Medicine* **Targeting Tumor Antigen Her2/Neu to Receptor Treml4 on Cd8α Dendritic Cells *In Situ* Induces Anti-Tumor Immunity**	
9:30 a.m. – 10:00 a.m.	**Nancy Andrews, MD, PhD** *Duke University* **The Secret Life of the Transferrin Receptor**	
9:30 a.m. – 11:00 a.m.	**APSA Social Science and Humanities Forum**	Embassy
10:00 a.m. – 11:00 a.m.	**APSA Infectious Disease Interest Group**	State
10:00 a.m. – 10:30 a.m.	Break	International Ballroom Foyer
10:30 a.m. – 11:00 a.m.	**Lennart Mucke, MD** *Gladstone Institute of Neurological Disease* **Brain Against Alzheimer's Disease: Rivals in Complexity**	International Ballroom
11:00 a.m. – 11:15 a.m.	**Laurence A. Turka, MD** *JCI Editor in Chief* **JCI 2012 — Handoff to the (Blue) Devil**	

Scientific Program

Saturday, April 28

Time	Event	Location
11:15 a.m. – 11:30 a.m.	**Oral Abstract Presentation** **John H. Lee, BS** *University of Iowa* **Investigating the Therapeutic Effect of Rhes Suppression in a Mouse Model of Huntington's Disease**	
11:30 a.m. – Noon	**Beth Levine, MD** *The University of Texas Southwestern Medical Center at Dallas* **Exercise, Autophagy, and Glucose Metabolism**	International Ballroom
Noon – 1:30 p.m.	Poster Session with Lunch	Imperial Ballroom
1:30 p.m. – 2:00 p.m.	Poster Dismantle	Imperial Ballroom
1:45 p.m. – 2:15 p.m. and 2:30 p.m. – 3:00 p.m.	**APSA Session – Career Development Workshop Sessions** **Residency 101 – Residency Options for Students Wishing to Pursue Careers as Physician Scientists** Moderator: Jaimo Ahn **David VanderWeele, MD, PhD,** *University of Chicago* **Vanessa Nomellini, MD, PhD,** *University of Wisconsin*	Gold
	Grant Writing & Funding - **Explanations of the NIH Grant Review Process** Moderator: Stephanie Jackson **M. Kerry O'Banion, MD, PhD** *University of Rochester Medical Center*	Embassy
	Journal Writing Moderator: Stephanie Jackson **Laurence Turka, MD,** *2007-2012 Editor-in-Chief of the* Journal of Clinical Investigation, *Harvard University* **Peter Wagner, MD,** *Editor-in-Chief,* Journal of Applied Physiology	State
1:30 p.m. – 3:00 p.m.	**ASCI and AAP New Member Presentations** Moderators: J. Larry Jameson, Peter Tontonoz and Taylor Heald-Sargent	International Ballroom
1:30 p.m. – 1:50 p.m.	**Joseph C. Wu, MD, PhD** *(New ASCI Member)* *Stanford University School of Medicine* **iPSC for Cardiac Disease Modeling and Cell Therapy**	
1:50 p.m. – 2:10 p.m	**Jean E. Schaffer, MD** *(New AAP Member)* *Washington University School of Medicine* **Unexpected Regulators of Metabolic Stress**	
2:10 p.m. – 2:30 p.m	**Rochelle P. Walensky, MD, MPH** *(New ASCI Member)* *Harvard Medical School, Massachusetts General Hospital* **HIV Care in the U.S. and Africa: From Cohorts and Trials to Policy**	
2:30 p.m. – 2:50 p.m.	**Joseph T. Bass, MD, PhD** *(New AAP Member)* *Northwestern University* **Biological Oscillators: From Behavior to Bioenergetics**	
3:00 p.m. – 3:30 p.m.	Break	International Ballroom Foyer

Scientific Program

Saturday, April 28

Time	Event	Location
3:30 p.m. – 4:00 p.m.	**APSA Keynote – Sanjiv Gambhir, MD, PhD** *Stanford University* **Bridging the Physical and Biological Sciences for Improving Patient Cancer Management**	International Ballroom
4:00 p.m. – 4:30 p.m.	**Myron S. Cohen, MD** *University of North Carolina, Chapel Hill* **AAS-Science Breakthrough of the Year 2011: How Treatment of HIV Became Prevention**	
4:30 p.m. – 4:45 p.m.	**APSA Trainee Presentation** **Catherine T. Jordan, BS** *Washington University in St. Louis* **Mutations in Card14 Lead to Psoriasis and Psoriatic Arthritis**	
4:45 p.m. – 5:15 p.m.	**APSA Awardee – Bert Shapiro, PhD** *Outgoing National Director, Medical Scientist Training Program, NIH* **A Student-friendly MSTP and a Call for Action**	
5:15 p.m. – 5:45 p.m.	**AAP Presidential Address: David Brenner, MD** **Next Gen Academic Medicine**	
5:15 p.m. – 6:15 p.m.	APSA Policy Panel	Gold Room
5:45 p.m. – 6:15 p.m.	**ASCI Presidential Address: Elizabeth McNally, MD, PhD** **Advocacy: Yes We Can**	International Ballroom
7:00 p.m. – 10:00 p.m.	**AAP Annual Reception and Dinner** **Connected: The Surprising Power of Our Social Networks and How They Shape Our Lives** Speaker: **James H. Fowler** *University of California, San Diego*	Imperial Ballroom and Foyer
7:30 p.m. – 9:00 p.m.	**APSA Dinner** Speaker: **Chi V. Dang, MD, PhD** *University of Pennsylvania*	Moulin Rouge
10:00 p.m. – Midnight	Dessert Reception (open to all attendees)	Imperial Ballroom Foyer

Scientific Program

Sunday, April 29

Time	Event	Location
7:00 a.m. – 10:00 a.m.	Registration	International Ballroom
7:00 a.m. – 8:15 a.m.	Continental Breakfast	Imperial Ballroom Foyer
7:00 a.m. – 8:15 a.m.	**Young Investigators' Mentoring Breakfast**	Moulin Rouge
8:15 a.m. – Noon	**Plenary Session – Understanding Disease Mechanisms to Improve Human Health** Moderators: David A. Brenner, Elizabeth McNally, and Ivayla I. Geneva	International Ballroom
8:15 a.m. – 8:30 a.m.	Best Poster Awards Presentation	
8:30 a.m. – 8:45 a.m.	AAP Business Meeting	
8:45 a.m. – 9:15 a.m.	**Kober Medal Presentation** Recipient: **Arthur H. Rubenstein, MBBCh** Presenter: **Kenneth Polonsky, MD**	
9:15 a.m. – 10:00 a.m.	**Kober Lecture** **Barry S. Coller, MD** *The Rockefeller University* **Translating from the Rivers of Babylon to the Coronary Bloodstream**	
10:00 a.m. – 10:15 a.m.	**APSA Trainee Presentation** **Muhammad K. Riaz, MD** *Jewish Hospital* **Prospective Assessment of Determinants of Apob, Apoa1, and the Apob/Apoa1 Ratio in 797 Healthy Schoolgirls, Studied from Mean Ages 10 to 19 Years: The Cincinnati National Growth and Health Study**	
10:15 a.m. – 11:00 a.m.	**ASCI/Stanley J. Korsmeyer Award Lecture** **William G. Kaelin, Jr., MD** *Dana-Farber Cancer Institute* **Gregg L. Semenza, MD, PhD** *Johns Hopkins Medicine*	
11:00 a.m. – 11:15 a.m.	**APSA Trainee Presentation** **Emily B. Heikamp, BS, MSc** *Johns Hopkins University School of Medicine* **Identification of Serum- and Glucocorticoid-Inducible Kinase 1 (Sgk1) as a Novel Regulator of Effector T Cell Differentiation**	
11:15 a.m. – 11:45 a.m.	**APSA Keynote – David Relman** *Stanford University* **Our Many, Varied Relationships with the Microbial World**	
11:45 a.m. – Noon	**APSA Trainee Presentation** **Natalia S. Chaimowitz, BS** *Virginia Commonwealth University* **Adam10 Regulates Plasma Cell Differentiation**	
12:15 p.m. – 1:00 p.m.	**Post Graduate Opportunities Panel**	International Ballroom
1:00 p.m. – 2:30 p.m.	Residency Luncheon	Moulin Rouge

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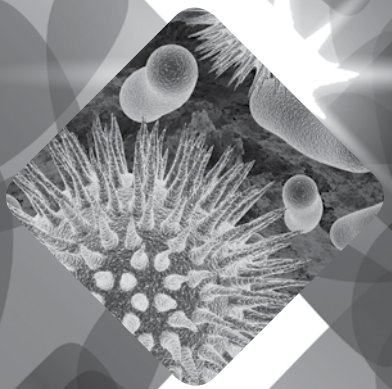
ASCI / AAP Joint Meeting

2012

Abstracts for Poster Session and Oral Presentations



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Poster Abstracts

1

TREK-1 Mediates the Development of Diastolic Dysfunction after Pressure Overload

D Abraham*, B Williams*, HA Rockman*†‡**
*Departments of *Medicine; †Cell Biology; and ‡Molecular Genetics, Duke University Medical Center, Durham, NC*

Background: Diastolic Heart Failure is a disease entity that is both highly prevalent and associated with significant morbidity. This disease is primarily caused by impaired relaxation and/or increased stiffness of the left ventricle, referred to as diastolic dysfunction. A consistent feature associated with diastolic dysfunction is pathologic hypertrophy, although the mechanistic relation between cardiac hypertrophy and diastolic heart failure remains uncertain. **Methods:** We tested the hypothesis that cardiac hypertrophic signaling pathways that are activated with pressure-overload can be distinguished from those that induce diastolic dysfunction. In a deficiency screen for cardiomyopathy in adult *Drosophila*, we identified a previously uncharacterized gene with homology to the mammalian two-pore domain K^+ channels, TREK-1. TREK-1 (TWIK-Related K^+ Channel-1) KO mice (n=21), which lack functional stretch activated two-pore domain K^+ channels, and wild type mice (n=6) underwent transverse aortic constriction (TAC) to induce pressure-overload hypertrophy. Serial conscious echocardiography was performed over a 16-week period. Hemodynamic analysis using a high fidelity LV micromanometer pressure catheter (Millar) was performed in mice 2 weeks after TAC. **Results:** TREK-1 KO animals developed exaggerated hypertrophy in comparison to wild type mice after 2 weeks of TAC, which was maintained over a 16 week period (Septal Wall Thickness 1.58mm ± 0.04 vs. 1.22mm ± 0.07, p<0.0001, (2-way ANOVA)). Remarkably, post TAC cardiac function remained normal at 16 weeks in TREK-1 KO mice compared to wild type mice (Fractional Shortening 52% ± 2 vs. 33% ± 7, p=0.044 (2-way ANOVA). Despite significant cardiac hypertrophy, hemodynamic analysis in TREK-1 KO mice showed normal diastolic function compared to wild type mice (dP/dtmin -9931 ± 382 mm Hg/sec vs. -6688 ± 270 mm Hg/sec, p=0.005 (t-test)) and significantly diminished fibrosis. **Conclusions:** Our data show that in response to pressure overload, loss of TREK-1 uncouples cardiac function phenotype from the pathological hypertrophic response. These findings support the hypothesis that the molecular mechanisms by which biomechanical signals are transduced into hypertrophic signaling pathways are distinct from those that induce diastolic dysfunction.

2

Pediatric Risk Factors and Changes Over 26 Years as Predictors of Adult Cardiovascular Disease and Impaired Fasting Glucose plus Type 2 Diabetes Mellitus: The Princeton Lrc Follow-Up Study

***C. Abuchaibe*[2], Charles J. Glueck[2], John A Morrison[1], Ping Wang[2], G. Boriel[2], J. Jurantee [2], X. Deng[2]**
[1]Division of Cardiology, Children's Hospital of Cincinnati; [2]Cholesterol and Metabolism Center, Jewish Hospital of Cincinnati

Objective: Assess pediatric risk factors and changes over 26 years as conjoint predictors of adult cardiovascular disease (CVD) and impaired fasting glucose (IFG) plus type 2 diabetes mellitus (T2DM). **Study Design:** 770 schoolchildren ages 5-20 (mean age 12), 26-yr prospective follow-up. Triglycerides (TG), LDL cholesterol, BMI, blood pressure and glucose ≥, and HDL cholesterol < pediatric and young adult cutoffs, race, cigarette smoking, family history of CVD and T2DM, and 26 year changes were assessed as predictors of CVD and IFG + T2DM. **Results:** Adult CVD (19 yes, 751 no) was independently and significantly associated with high TG at both ages 12 and 39 (p=.0005). Age at follow-up was positively associated with CVD (p=.0009), and low BMI at both ages 12 and 39 was inversely associated with CVD (p=.02). Adult IFG + T2DM (125 yes, 576 no) was associated with adult high blood pressure (p=.003), BMI (p=.001), and TG (p=.008), with high pediatric glucose (p=.0005), with TG high at both ages 12 and 39 (p=.049), and with parental T2DM (p=.012). **Conclusions:** Pediatric and adult risk factors predict CVD and IFG+T2DM at mean age 39; adult risk factors do not attenuate those in childhood.

Supported by American Heart Association (National) 9750129N and NIH-HL62394 (Dr Morrison), Lipoprotein Research Fund of the Jewish Hospital of Cincinnati (Dr Glueck).

3

Antibody-Induced Lysosomal Cell Death is Mediated Through a Novel Reactive Oxygen Species Dependent Pathway in Human Lymphoma and Leukemia Cells

***Alduaij W*[1], Honeychurch J[1], Azizyan M[1], Cheadle EJ [1], Pelicano H[2], Ivanov A[3], Cragg MS[4], Huang P[2] and Illidge TM[1]**
[1] Targeted Therapy Group, Paterson Institute for Cancer Research, School of Cancer and Enabling Sciences, University of Manchester, Manchester Academic Health Sciences Centre, United Kingdom [2] Department of Molecular Pathology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA. [3] Beatson Institute for Cancer Research, Glasgow, United Kingdom. [4] Tenovus Laboratory, Cancer Sciences Unit, University of Southampton Faculty of Medicine, Southampton, United Kingdom

Monoclonal antibodies (mAbs) have revolutionized the treatment of B-cell malignancies. Although Fc-dependent mechanisms of mAb-mediated tumor clearance have been extensively studied, the ability of mAbs to directly evoke programmed cell death (PCD) in the target cell and the underlying mechanisms involved remain under-investigated. We recently demonstrated that certain mAbs (specifically type II anti-CD20 and anti-HLA DR mAbs) potently evoked PCD through an actin-dependent, lysosome-mediated process (Ivanov et al *J Clin Invest* 2009, Alduaij et al *Blood* 2011). In this study, we reveal that the induction of PCD by these mAbs, including the novel type II anti-CD20 mAb GA101, directly correlates with their ability to produce reactive oxygen species (ROS) in human B-lymphoma cell lines and primary B-cell chronic lymphocytic leukemia cells. ROS scavengers abrogated mAb-induced PCD indicating that ROS are required for the execution of cell death. ROS were generated downstream of the mAb-induced actin cytoskeletal reorganization and lysosome membrane permeabilization. ROS production was independent of mitochondria and unaffected by BCL-2 overexpression. Instead, ROS generation was mediated by NADPH oxidase. These findings provide further insights into a previously unrecognized role for NADPH oxidase-derived ROS in mediating non-apoptotic

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PCD evoked by mAbs in B-cell malignancies. This newly characterized cell death pathway may potentially be exploited to eliminate malignant B-cells which are refractory to conventional chemotherapy and immunotherapy.

4 (Cancelled)

Case Report: A Case of Acute Kidney Injury Following the Use of Anabolic Androgenic Steroids

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Introduction: Body satisfaction and body mass index (BMI) have been demonstrated as key factors affecting self-esteem. People who are dissatisfied with their body image tend to misuse drugs and exhibit an unhealthy behavior to enhance their body image. Anabolic Androgenic steroids (AAS) is one of these drugs and have been abused by general public to enhance body image and thus became a public health problem. AAS have an adverse effect on mental, physical, and sexual wellbeing but more seriously an acute kidney injury. The case we report here describes an acute kidney injury due to AAS abuse as a result of hypercalcemia. **Case presentation:** a 32 year-old white male complaining of body aches, malaise and weakness concomitant with abdominal pain, nausea and vomiting. Initial laboratory investigations showed severe hypercalcemia and renal impairment. **Conclusion:** Although acute kidney injury is a rare side effect of AAS it is likely to increase with increasing number of people using AAS. This case in addition to other renal, cardiovascular, reproductive, endocrine, hepatic, dermatological and psychiatric side effects of AAS, highlight the importance of increasing awareness about AAS side effect among general public.

5 (Cancelled)

Assessing Human Circadian Phase through Transcriptional Profiling

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Introduction: Measuring the entrained circadian phase of human subjects is laborious and costly. As part of a larger study on the effects of sleep deprivation on leukocyte transcription, we identified transcripts that have a strong circadian pattern and are unaffected by recent sleep history. We tested our ability to estimate the circadian phase of human subjects using a limited panel of these transcripts. **Methods:** Fourteen subjects (ages 19 to 44) were instructed to follow a regular schedule for two weeks prior to their admission to the clinical research center. Blood samples were collected every 4 hours during an uninterrupted sleep/wake cycle and during a voluntary 38-hour period of acute sleep deprivation. Total RNA was extracted and transcript levels were measured using a custom human GeneChip® microarray. We identified diurnally regulated transcripts using both COSINOR analysis and the JTK_CYCLE algorithm with a false discovery rate cutoff of 1%. We then developed a support vector machine (SVM) model (libsvm, R programming environment) to predict the time of sample collection given a panel of transcript levels. The accuracy of these predictions was assessed through cross-validation. **Results:** Nearly 5000 transcripts were identified as cycling, with a greater than 80% overlap in the transcripts identified by the two methods. The vast majority of these continued to cycle, unaffected, despite acute sleep deprivation. However, inter-subject variation of mean transcript levels limited the utility of a single blood draw for phase assessment. Using a panel of 50 transcripts and two blood draws separated by 8 hours, the SVM model was able to correctly classify the phase of a subject with an accuracy of 92%. **Conclusions**. This study indicates that measurement of transcript levels from peripheral blood samples at two time points may be a promising and cost effective means of assessing circadian phase.

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Immune Dysregulation in Murine Sickle Cell Disease is Associated with Altered Splenic Architecture and Exaggerated Sensitivity to Vaccination

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Sickle Cell Disease (SCD) is caused by an autosomal recessive mutation of the beta-globin gene leading to sickling of erythrocytes and splenic auto-infarction. SCD patients are prone to infectious diseases; however, the reasons for this relative immunodeficiency have not been fully elucidated. Furthermore, how changes in baseline immunity affect the mechanism of action of vaccines in SCD is not well understood. **Objectives:** We utilized a SCD mouse model to evaluate baseline differences in immunity and to correlate such changes with responses to vaccination. **Methods:** Humanized transgenic α- and β^s-globin SCD mice and C57BL/6 mice were sacrificed either at baseline or after vaccination with ovalbumin (OVA)/Alum. Vaccination was administered intraperitoneally (IP) or intramuscularly(IM) weekly x3 weeks. Spleens were harvested and examined by histopathology and confocal microscopy. Immunophenotyping of splenic leukocytes was conducted using flow cytometry. Blood was collected by cardiac puncture and serum collected. Serum immunoglobulin and cytokine concentrations were determined using Luminex bead assays. Serum OVA-specific IgE was measured by ELISA. **Results:** Analysis of splenic tissue from naïve SCD mice revealed severely disrupted microarchitecture with attenuation of white pulp and no discernable T-cell or marginal zones. Immunophenotyping of splenic lymphocytes from naïve SCD mice demonstrated the presence of a disproportionately high percentage of "marginal zone" B-cells, possibly compensating for loss of other cell types in this organ. Both IP and IM vaccination resulted in high levels of mortality in SCD mice (45-50%) not observed in wildtype mice. Significant lower serum IL-1α, RANTES, and IP-10 were observed prior to vaccination in SCD mice, while IL-6 was increased. After vaccination, IFN-γ, GM-CSF, IL-1α, IL-2, IL-5, and MIP-1α were

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found to be lower in SCD mice. Surprisingly, post-vaccination OVA-specific serum IgE concentrations were 10-fold higher in SCD mice compared to C57/BL6. **Conclusions:** Baseline immunologic changes observed in SCD mice likely contribute to exaggerated sensitivity to vaccination, which is supported by significantly increased serum levels of OVA-specific IgE in vaccinated SCD mice. These results indicate that SCD is a state of dysregulated immunity that may be the result of abnormal splenic function which may contribute to the profound susceptibility to infection.

7

A Tbx5-Scn5a Molecular Network Modulates Function of the Adult Cardiac Conduction System

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Cardiac conduction system (CCS) disease is common with significant morbidity and mortality. Current treatment options are limited and rational efforts to develop cell-based and regenerative therapies require knowledge of the molecular networks establishing and maintaining CCS function. Recent genome wide association studies (GWAS) have identified numerous loci associated with adult human CCS function, including *TBX5* and *SCN5A*. We hypothesized that *Tbx5*, a critical developmental transcription factor, regulates transcriptional networks required for mature CCS function. Removal of *Tbx5* from the mature murine ventricular conduction system (VCS), including the AV bundle and left and right bundle branches, resulted in a loss of fast conduction, cardiac arrhythmias, and sudden death. Ventricular contractile function and the VCS fate map remained unchanged in VCS-specific *Tbx5* knockouts. However, key mediators of fast conduction including Cx40 and Nav1.5, encoded by *Scn5a*, demonstrated Tbx5-dependent VCS-specific expression. We identified a Tbx5-responsive enhancer downstream of Scn5a sufficient to drive in-vivo VCS expression, dependent on canonical T-box binding sites. Our results establish a direct molecular link between Tbx5 and *Scn5a* and establish a molecular hierarchy between human GWAS loci that drives VCS function in the mature CCS, establishing a paradigm for understanding the molecular pathology of VCS disease. Future studies are focusing on dissecting the regulation of *Scn5a* expression using a Scn5a-LacZ BAC transgenic reporter system. We anticipate that these studies will lend important molecular insights into understanding the regulation of *Scn5a*.

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PACS-2 Is a Novel Regulator of Sirtuin 1-Mediated Deacetylation of P53 Following DNA Damage

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Sirtuin 1 (Sirt1) is an NAD^+-dependent deacetylase that has been implicated in energy metabolism, cell death/survival, replicative senescence, tumorigenesis, as well as other cellular processes. While its substrates, such as the tumor suppressor p53, have been well characterized, very few regulators of Sirt1 are currently known. Here we describe the multi-functional trafficking protein PACS-2 as a novel regulator of Sirt1. PACS-2 antagonizes Sirt1-mediated deacetylation of p53 leading to increased levels of acetylated p53 (ac-p53). Indeed, post-translational modification of p53, such as phosphorylation and acetylation, is essential for its activation and transcription factor role, enabling p53 to bind promoters and modulate the DNA damage response by inducing the transcription of various target genes leading to cell cycle arrest (p21, GADD45), apoptosis (Puma, Bax) or senescence (p21). Here, we determined that p53 molecules in cells from PACS-$2^{-/-}$ mice have reduced acetylation of p53 following ionizing radiation (IR). Consistent with this finding, HCT116 colon cancer cells depleted of PACS-2 have reduced acetylation of p53 following treatment with the chemotherapeutic doxorubicin, but not when treated in combination with the Sirt1 inhibitor EX527, suggesting the decreased acetylation of p53 in PACS-2-depleted cells is Sirt1-dependent. Moreover, PACS-2 knockdown HCT116 cells have reduced levels of acetylated p53 on the p21 promoter following DNA damage by chromatin immunoprecipitation analysis. This correlates with reduced transcriptional output of p53 as well, as the expression levels of the p53 target genes p21 and Puma are also reduced in PACS-2 knockdown HCT116 cells and in tissues from PACS-$2^{-/-}$ mice following DNA damage. Further analysis revealed that PACS-2-depleted cells have accelerated apoptosis and reduced clonogenic survival following DNA damage, suggesting PACS-2 is a novel regulator of the DNA damage response in vivo. In response to lethal doses of IR, mice deficient in p53 or that fail to induce p53-mediated apoptosis are resistant to death from hematopoietic (HP) syndrome. Indeed, PACS-$2^{-/-}$ mice are sensitized to lethal doses of IR that induce HP syndrome. Together, these data suggest that PACS-2 modulates p53 action following DNA damage by antagonizing Sirt1-mediated deacetylation of p53, and therefore, the ability of p53 to effectively respond to DNA damage.

9

The Lineage-Specific Transcription Factor ASCL1 Defines a Subtype of Non-Small Cell Lung Cancer with Poor Prognosis and Points to Potential Therapeutic Targets

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Introduction: Using genome-wide expression analyses several groups have identified a neuroendocrine subtype of NSCLC (NE-NSCLC), occurring in 5-8% of all NSCLC, which has a poorer prognosis than typical NSCLC. To determine a preclinical model for NE-NSCLC, we utilized whole-genome mRNA expression array data from 119 NSCLC cell lines to identify a class of NSCLC lines that fit the neuroendocrine phenotype. A gene expressed in all putative NE-NSCLC cell lines is the potent neural-specific transcription factor ASCL1. We found that ASCL1 is: necessary for the survival of NE-NSCLC cell lines; correlates with stem cell marker expression; and that an ASCL1-associated gene signature

predicts for poor prognosis in NSCLC. **Aims and Methods:** We hypothesize that ASCL1 acts as a "lineage dependent oncogene" for NE-NSCLC and that associated with ASCL1's function in the molecular pathogenesis of these lung cancers will be a gene expression profile that contributes to the malignant phenotype, which will provide insight towards therapeutic targeting of this subset of NSCLC. To test this hypothesis we examined genome wide mRNA expression data from 119 NSCLC lines with validation of selected genes by qRT-PCR, gathered ASCL1 ChIP-Seq data using ASCL1 expressing NSCLC lines, determined the clinical outcome of 275 resected NSCLCs characterized for ASCL1 and genome wide mRNA expression, and performed functional analyses in NSCLC lines with siRNA and inducible shRNA knockdown of ASCL1. **Results and Conclusions:** We identified 11/119 NSCLC cell lines (9.2%) that display a distinct neuroendocrine gene signature. Knockdown of ASCL1 in representative NSCLC lines reduced target gene mRNA, caused significant cell cycle defects, and induced apoptosis. ASCL1 ChIP-Seq data combined with genome wide mRNA expression data identified a subset of genes that appear to be regulated by ASCL1 and a prediction-based algorithm was used to show that expression of ASCL1-regulated genes correlates with impaired prognosis. Additionally, NSCLC cell lines expressing ASCL1 demonstrate a cancer stem cell marker phenotype similar to that of small cell lung cancer. Our results suggest that neuroendocrine gene expression in NSCLC is of clinical relevance, that ASCL1 is required for survival of the NE-NSCLC disease subset, while the integrated ASCL1 ChIP-Seq and mRNA expression data provide a roadmap for systematically searching for therapeutic targets for this phenotype and their mechanistic role in a cancer stem cell (initiating cell) subpopulation within these tumors.

10 (Cancelled)

Identifying the Role of TULA-2 a Novel Tyrosine-Phosphatase in Bone Remodeling

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Hematopoietic stem cells (HSC) are committed to the osteoclast lineage when the cell surface receptors, c-FMS and RANK, are activated by their ligands MCSF and RANKL respectively. Additionally, other co-stimulatory receptors are necessary to ensure proper osteoclast differentiation. Two of these receptors, OSCAR and TREM, are transmembrane receptors that contain immunotyrosine activation motifs (ITAM), termed FcRγ and DAP12 respectively, which exist on the cytoplasmic tail of their respective receptors. Once these receptors are triggered, specific tyrosines in the ITAM motifs become phosphorylated and act as docking points for Syk tyrosine kinase. Once docked, Syk autophosphorylates and acts on downstream targets. Syk dephosphorylation is necessary to attenuate this signal to prevent over activation of osteoclasts. Recently, a novel tyrosine phosphatase, T-cell Ubiquitan ligand -2 (TULA-2) has been shown to dephosphorylate specific phosphotyrosine residues on Syk. The goal of our project is to determine how TULA-2 mediated dephosphorylation of Syk regulates osteoclast differentiation and function. TULA-2 is a member of the TULA family of proteins, TULA and TULA-2. In spite of a significant homology and similar domain organization between TULA and TULA-2, only TULA-2 has significant phosphatase activity. Furthermore, whereas TULA is expressed only in lymphocytes, TULA-2 is expressed in most tissues including cells of hematopoietic origin. *In vivo* analysis including x-ray, histomorphometry and uCT indicated that mice that lack both TULA and TULA-2 (DKO) have decreased bone mass compared to wild-type (WT) counterparts. *In vitro* cell differentiation assay indicated that osteoclast-like cells cultured from DKO bone marrow were more numerous when compared to WT. Additionally, an *in vitro* resorption pit assay revealed that DKO OCL could resorb bone at a faster rate than WT counterparts. At the molecular level, a probe for total tyrosine phosphorylation after FcR and c-fms stimulation of bone marrow macrophages (BMM) revealed increased phosphotyrosines at various molecular weights in DKO BMM when compared to WT BMM. A probe for phosphorylation of Syk revealed increased phosphorylation at tyrosine 346 in DKO osteoclasts when compared to WT osteoclasts. Cumulatively, the above data indicates that the absence of TULA-2 results in an increased signaling response leading to more numerous, hyperactive osteoclasts which contributes to decreased bone mass in mice suggesting that the phosphatase activity of TULA 2 is required for negative regulation of bone resorption.

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Impaired Microtubular Transport in Tauopathy Leading to Autophagy Gridlock

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Tauopathies are a class of neurodegenerative diseases characterized by the presence of tau aggregates in the neurons. Examples are Alzheimer disease, progressive supranuclear palsy and frontotemporal dementia linked to chromosome 17. In a previously developed Drosophila model of tauopathy, expression of human tau under an eye-specific promoter produced a dosage-sensitive rough eye phenotype. Here we show that misexpression of human tau in the fly eye colocalizes with Drosophila tubulin, an essential microtubules protein. Tau misexpression induced the formation of acidic punctae in the developing eye disc which colocalized with Atg5, suggesting that these vesicles are autophagic vacuoles. Ultrastructure analysis of the adult eye using transmission electron microscopy showed that tau induced the formation of double membrane vesicles which we identified as autophagosomes. Reduction in the endogenous levels of kinesin heavy chain resulted in the enhancement of the toxic phenotype. Moreover, we observed the formation of large tau aggregation spheres that were surrounded by autophagic markers such as Atg5, Atg8 and ALFY and contained LAMP-2 and actin in addition to tau. We suspect that these giant aggregates may be similar to granuolovacuolar degeneration (GVD) bodies which are similar in size and are also rich in late autophagic markers. GVD bodies are a hallmark of late stage AD brains. Additionally, we found that players in the selective autophagy, involved in aggregates clearance such as HDAC6 and blue cheese (Drosophila homologue of ALFY), are modifiers of tau toxicity *in vivo*. We also show that autophagy activation through either environmental, chemical or genetic stimuli results in the suppression of tau-induced toxicity.

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Caloric restriction causes reduction of the rough eye phenotype by 56%. Moreover, induction of autophagy by feeding flies on rapamycin, an inhibitor of Target Of Rapamycin also results in suppression of the tau-induced roughness. Genetic upregulation of key autophagic and lysosomal genes such as Atg2, Atg7 and cathepsinD also suppress the rough phenotype, whereas downregulation of key autophagic genes, such as Atg1, Atg4 and Atg6 results in the enhancement of the rough phenotype. In summary, we demonstrate that the early activation of autophagy suppresses tau toxicity, and that tau induces autophagy, yet the autophagic flux is impaired as seen by the accumulation of autophagosomes and large aggregates containing autophagic markers. These findings provide insight into the mechanism by which tau mediates cytotoxicity in neurodegenerative diseases.

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Examining the Selective Contribution of Chromosomal Instability to Tumor Evolution and Prognosis

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The specific role of Chromosomal Instability (CIN) in tumorigenesis has been a matter of conjecture. In part, this is due to the challenge of directly observing dynamic whole-chromosome mis-segregation events as well as the lack of firm understanding of the mechanisms that lead to CIN in cancer. These shortcomings have long prevented the ability to distinguish the role of CIN, which consists of increased 'rates' of chromosome mis-segregation, from that of aneuploidy, which is a 'state' of non-diploid chromosome number. Here, we show that chromosome mis-segregation in cancer-derived cell lines results from defective microtubule dynamics. This defect compromises the correction of erroneous attachments of microtubules to chromosomes during mitosis, which leads to the formation of lagging chromosomes and chromatin bridges during anaphase. Strikingly, restoring normal microtubule dynamics significantly decreases chromosome mis-segregation frequencies and suppresses CIN. We then use lagging chromosomes and chromatin bridges as morphological features to examine the selective contribution of CIN to tumor prognosis. H&E-stained samples from 54 patients diagnosed with Diffuse Large B-Cell Lymphoma (DLBCL) were used to examine the relationship between frequencies of chromosome mis-segregation and patient prognosis, overall survival, and response to treatment. We show that a two-fold increase in the frequency of chromosome mis-segregation leads to a 24% decrease in overall survival and 48% decrease in relapse-free survival after treatment. The hazard ratio (HR) of death in patients with increased chromosome mis-segregation is 2.31 and these patients are more likely to present with higher tumor stage, exhibit tumor bone marrow involvement, and receive a higher International Prognostic Index (IPI) score. In summary, this work demonstrates that CIN primarily arises due to defects in the attachments of microtubule to chromosomes and that increased rates of chromosome mis-segregation may substantiate inferior outcome and poor prognosis. We propose that targeting CIN by therapeutically altering microtubule dynamics would yield improved prognosis and enhanced response to chemotherapeutic drugs.

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Cadherin Point Mutations Alter Binding Affinities and Modulate Rac1 Signaling

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Cadherins are cell-cell adhesion proteins that mediate cell recognition, segregation, and signaling in many tissues. Compromised cadherin activity alters Rho GTPase signaling and is associated with a diverse range of diseases including metastatic cancer. In addition, mutations in the extracellular domains of cadherins have been identified in cardiac, retina, and skin disorders. Thus, we investigated the implications of cadherin binding-site mutations on cadherin binding affinities and ligation-dependent Rac1 signaling. Stable cell lines were engineered to stably express point mutations in the first extracellular domain (EC1) of C-cadherin. Using micropipette manipulation, we quantified two-dimensional binding affinities of both WT cadherin and the point mutants in the native environment of the cell membrane. These mutations induced up to a six fold difference in binding affinities. Further studies compared these binding affinities with ligation-activated Rac1 signaling. Dynamic fluorescence imaging examined Rac1 activation at nascent cell-cell junctions, based on the accumulation of a fluorescent Rac1 reporter at the junctions. Dynamic fluorescence imaging revealed qualitative correlations between the measured affinities and the signal amplitude triggered by cadherin ligation. These measurements were also supported by immuno pull-down assays of GTPase activity. These findings establish an important link between cadherin binding-site mutations and quantitative differences in affinity, adhesion, and signaling. Diminished cadherin function may lead to structural and functional defects in tissues and organs, contributing to disease progression. Because cadherins and their effectors are promising therapeutic targets, understanding the biochemical and biophysical mechanisms underlying cadherin adhesion and signaling in disease is critical.

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Twice Daily Dosing of Indomethacin is More Effective in Patent Ductus Arteriosus Closure Compared to Daily Dosing of Indomethacin or Ibuprofen

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Objective: To determine whether ibuprofen or indomethacin, and which dosing interval of indomethacin, is most effective in achieving closure of a patent ductus arteriosus (PDA), and if there is a difference in complication rates. **Study Design:** 227 infants were retrospectively reviewed and divided into 1 of 3 treatment groups: ibuprofen, daily and twice daily indomethacin. One course of treatment was given over 3 days. Chi-squared and Fisher's Exact analyses were used. **Results:** Twice daily indomethacin was significantly more efficacious in closing PDA as compared to

either daily indomethacin or ibuprofen (75% closure rate vs 53% and 60% respectively; p=0.02). There was no significant difference in gastrointestinal perforation, bronchopulmonary dysplasia, necrotizing enterocolitis, intraventricular hemorrhage, or mortality. **Conclusion:** A 6 dose course of twice daily dosing of indomethacin is significantly more effective at achieving PDA closure compared to a 3 dose course of daily dosing of either indomethacin or ibuprofen, with no significant difference in complications. **Table 1:** Outcome in the three treatment groups

	Ibuprofen n=98 (%)	Twice daily indomethacin n=99 (%)	Daily indomethacin n=30 (%)	P-value
PDA closure	59 (60)	75 (76)	16 (53)	**0.02**
Spontaneous GI perforation	7 (7.1)	7 (7.1)	1 (3.3)	0.87
NEC (grade 2 or 3)	10 (10.2)	15 (15.2)	1 (3.3)	0.21
BPD	76/93 (81.7)	68/91 (74.7)	25/29 (86.2)	0.36
IVH (all grades)	6/20 (30)	14/40 (35)	N/A	0.78
Mortality	7/90 (7.8)	16/96 (16.7)	2/24 (8.3)	0.15

Abbreviations: PDA, patent ductus arteriosus; GI, gastrointestinal; NEC, necrotizing enterocolitis; BPD, bronchopulmonary dysplasia; IVH intraventricular hemorrhage.

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CD56 T Cell Therapy For Multiple Myeloma

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Chimeric antigen receptors (CARs) that redirect T cell specificity to a cell surface expressed tumor antigen show considerable promise for the treatment of a number of malignancies. We have developed a novel adoptive immunotherapy strategy for multiple myeloma using a CAR targeted against CD56. CD56 is strongly expressed by malignant plasma cells in 70-80% of patients with myeloma at all stages of the disease with its role thought to be to facilitate the anchorage between myeloma cells and bone marrow stromal cells. CD56 is also expressed at lower levels on normal tissue including neuronal cells, NK cells and a subset of activated T cells. Phase I trials with the humanized anti-CD56 antibody N901 have shown no significant toxicity suggesting that targeting CD56 may be tolerable. A CD56 CAR was constructed containing an anti-hCD56 scFv linked to the CD28 transmembrane and CD3 zeta chain cytoplasmic signaling domains and expressed in human T cells by retroviral transduction. CD56 CARs showed antigen dependent proliferation and cytokine secretion *in vitro* and importantly although CD56+ve T cells were eliminated by the CAR, the CD56-ve fraction was able to proliferate with no functional impairment. CD56 CARs were able to effectively lyse CD56 positive myeloma cell lines *in vitro* with 40-60% specific lysis at E:T ratios >5:1. To further assess the antitumor activity of CD56 CARs *in vivo* we developed a systemic xenograft model of myeloma by injecting the OPM2 myeloma cell line expressing the firefly luciferase gene, intravenously into NOD/SCID Il2rγ^{null} mice. Bioluminescence imaging showed tumor progression predominantly within the bone marrow recapitulating the human disease phenotype with hind limb paralysis eventually occurring at 35 days following injection of $3x10^6$ tumor cells. OPM2 injected mice were treated with a single infusion of CD56 CARs at varying doses once tumor was established at 7 days. All mice receiving a dose $1x10^6$ CAR+ve cells showed complete tumor regression and remained tumor free at 3 months in contrast with control CAR treated mice all of whom developed tumor progression with a median survival of 34 days. CD56 CAR+ve cells were detected in the peripheral blood of these mice for upto 3 months. Significantly when cured mice were subsequently rechallenged with OPM2 tumor, a marked expansion of CAR+ve cells occurred leading to tumor rejection. These results demonstrate for the first time the impressive anti-tumor efficacy of a CD56 targeted chimeric antigen receptor in a systemic xenograft model of myeloma and support the need for further evaluation of its clinical efficacy and toxicity in a future phase I trial in patients with relapsed refractory myeloma.

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Translational Approaches for Delivery of Novel Activators of the Cerebroprotective ACE2/ Angiotensin-(1-7)/Mas System in Stroke

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In the United States, it is estimated that as many as 1 in every 6 people will suffer from a stroke, and approximately 1 in every 5 of these victims will suffer permanent disability. The pathophysiology of stroke is multifactorial, and the renin/angiotensin system (RAS) has been under investigation as a target for therapeutic intervention. There is mounting evidence that a detrimental axis of the RAS is activated during ischemic stroke. In contrast, activation of the newly discovered angiotensin converting enzyme 2/angiotensin-(1-7)/Mas (ACE2/Ang-(1-7)/Mas) axis, a counter-regulatory axis of the RAS, is neuroprotective in a rat model of ischemic stroke. Until now, activation of the ACE2/Ang-(1-7)/Mas axis has proven difficult, requiring direct intracerebroventricular (ICV) infusion of Ang-(1-7) peptides. An essential step in determining the feasibility of stroke therapy by activation of this neuroprotective pathway is to test novel means of compound administration that are translatable for use in treating human stroke patients. Here, we use two such minimally invasive therapies designed for use in the acute phase of ischemic stroke: 1 - peripheral drug injections of diminazene aceturate (DIZE), a novel ACE2 activator, and 2 - targeted delivery of lenti-viral infected Ang-(1-7) secreting Hematopoietic Stem Cells (HSCs), which have been shown to home to the site of brain infarct. We tested the hypothesis that activation of the brain's ACE2/Ang-(1-7)/Mas axis during ischemic stroke by clinically translatable methods of peripheral administration of novel compounds will result in cerebroprotection as determined by reduction in infarct size, and improvement in neurological deficits. Our data indicate that intraperitoneal injections of DIZE result in significantly decreased infarct size and improvement of neurologic deficits in an endothelin-1 (ET-1) induced middle cerebral artery occlusion (MCAO) model of ischemic stroke in rats. We also show significant reduction in neurologic deficits following injection of virally infected HPCs. These findings represent an important step in the translation of a rapidly advancing field of scientific inquiry

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centered on the brain's endogenous angiotensin systems into bedside therapeutics for stroke victims who largely suffer from a dearth of efficacious therapies.

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Genetic Variation in Gene Expression Response to Endoplasmic Reticulum Stress in Human B-cells

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Endoplasmic recticulum (ER) stress is caused by excessive demands on the protein-processing capacity of the ER; inefficiencies in response to ER stress lead to various human diseases including rheumatic conditions such as ankylosing spondylitis (AS). In this project, to study the role of ER stress in disease susceptibility, we identified the genes and pathways involved in ER stress response in human cells, assessed individual variation in these pathways, and mapped DNA sequence variants that influence ER stress response. While ER stress is well characterized in yeast and other model organisms, less is known in human cells. To begin, we studied ER stress response in human B-cells, which play key roles in inflammatory conditions, including AS. We exposed cultured B-cells from 130 normal individuals to tunicamycin, which induces ER stress by inhibition of N-glycosylation, for 8 hours. Then, using microarrays, we measured expression levels of genes and identified 1,523 genes whose expression levels changed significantly following ER stress induction. Among those that increased expression were genes involved in protein folding in the ER, such as *HSPA5*, which encodes the chaperone protein BiP. Genes that decreased expression were significantly enriched for those involved in DNA replication and RNA processing, suggesting that reallocation of limited cellular resources is important in response of human B-cells to ER stress. In addition, the gene expression results from the 130 individuals reveal significant individual differences in ER stress response. For example, genes that encode canonical ER stress sensors IRE1 and PERK vary by 4-fold and 3-fold, respectively, among our subjects. *HSPA5*, which also plays a key role in sensing ER stress, varies by 10-fold among our subjects. Individual differences are also seen in genes encoding transcription factors that have important roles in ER stress response, such as *XBP1* and *DDIT3* (CHOP), which vary by 5-fold and 9-fold, respectively. These results suggest that variation exists not only in sensing of ER stress but also in the extent of ER stress response. To understand the basis of individual differences in ER stress response, we carried out a genetic mapping study in B-cells from members of 15 large families. We measured gene expression responses to ER stress and treated them as quantitative phenotypes in genetic linkage and association studies. We focused our analysis on 778 ER stress-responsive genes and identified DNA sequence variants that regulate the response of 497 of the genes. These results allowed us to identify genes and their regulators involved in ER stress response in human B-cells. In this presentation, I will discuss our findings and their implications on susceptibility to ER stress-related diseases

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Progress in Underrepresented Minority Medical Student Recruitment: Successful Strategies, from Minority Students' Perspectives

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Background: In order to increase access to healthcare for the growing underserved populations of the United States, the American Association of Medical Colleges (AAMC) has called for an increase in underrepresented minority student enrollment into medical school. However, US medical schools have struggled to increase their class diversity. Few studies have focused on why minority applicants choose to attend one medical school versus another. **Purpose:** To assess the factors which made the greatest impact on underrepresented minority (URM) students' choice to attend Loyola University Stritch School of Medicine (LUSSOM) versus other US medical schools over the past 10 years, in which LUSSOM has seen an increase in URM student prevalence from 5% to 13% of their entering classes. **Methods:** We developed a survey in which various URM recruitment strategies utilized by LUSSOM were rated based on the impact which they made on URM students' decision to attend LUSSOM versus other medical schools. This survey was sent to all URM students who attended LUSSOM from 2001-2011. URM was defined by the AAMC as African American, American Indian/Alaskan Native, Mainland Puerto Rican, and Mexican American. **Results:** We are still in the process of collecting and analyzing the results of this study. **Conclusions:** Have yet to be made.

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Among HCV-infected Patients on Triple Therapy, Serum Calcium Is a New Indicator of Treatment Response

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Background: Ribavirin is an essential component of triple therapy for HCV infection and is used in combination with peg-interferon and a viral protease inhibitor. Ribavirin has minimal impact on HCV viral load. Therefore, alternative approaches are needed to monitor ribavirin's effectiveness during HCV treatment. Ribavirin decreases serum calcium in patients with respiratory infections. **Aim:** To assess serum calcium as a marker of ribavirin's biological effects in HCV-infected patients on triple therapy. **Methods:** Data of 69 patients with genotype 1 HCV receiving triple therapy with telaprevir at Mount Sinai were analyzed retrospectively with IRB approval. The Roche Ampliprep test was used to classify responses at week-4 into 3 categories: (1) undetectable HCV RNA, signifying a rapid virological response (RVR; N=32), (2) HCV < 43 IU/mL, signifying the need for at least 48 weeks of treatment (n=27), and (3) HCV ≥ 43 IU/mL (n=10). **Results:** The group was 63% white/non-Hispanic, with a median age of 57 years. At baseline, serum calcium was similar in the three response groups. At week-4, however, corrected calcium was significantly lower in RVRs than

in patients with HCV RNA ≥ 43 IU/mL (n=10, p=0.018). Corrected calcium decreased by 0.59 ± 0.08 mg/dL in RVRs versus 0.24 ± 0.15 mg/dL in patients with HCV RNA ≥ 43 IU/mL (Group 3). Calcium decreased by 0.56 ± 0.13 mg/dL in patients with HCV RNA < 43 IU/mL. The change in uncorrected calcium was significantly associated with the change in hemoglobin, a known marker of ribavirin's effects (Pearson r = 0.30, p=0.02). **Conclusions:** Serum calcium decreased during triple therapy. At week-4, the decrease was greatest among RVRs and smallest among patients with HCV RNA ≥ 43 IU/mL. Serum calcium may be a useful systemic marker of host responsiveness to ribavirin, similar to hemoglobin. Assuming that the decrease in calcium is a side effect that indicates the biological activity of triple therapy, patients who do not experience a decrease in serum calcium may have sub-optimal drug levels and might benefit from high drug levels, if this can be tolerated safely.

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Identification of Critical Residues within MLL Fusion Proteins that Contribute to Leukemia Development

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The *Mixed Lineage Leukemia* (*MLL*) gene can participate in chromosomal translocations which give rise to either acute myeloid or lymphoid leukemia. *MLL*-associated leukemias have a poor prognosis with 5-year survival rates of only 30-40%. The fact that these aggressive leukemias are dependent on the fusion protein suggests that the resultant MLL fusion protein would be an effective target for the development of new therapies. The aim of this study was to identify specific amino acids within a critical DNA-binding domain of MLL—the CXXC domain—which contribute to the leukemogenic capacity of MLL fusion proteins. The closest homolog of MLL—MLL2—also contains a similar CXXC domain, yet an artificial MLL2 fusion protein is unable to transform cells *in vitro*. Knowing that the CXXC domain is a critical component of MLL fusion proteins, we hypothesized that specific amino acid differences between the MLL and MLL2 CXXC domains account for differences in leukemogenic capacity. To test this hypothesis, we substituted the MLL2 CXXC domain for the MLL CXXC domain in the context of the MLL-AF9 fusion oncoprotein to generate an artificial MLL/MLL2-AF9 chimera. Amino acid substitutions within the MLL2 CXXC domain were then introduced to restore residues to the MLL amino acid sequence. Retroviral transduction of bone marrow progenitor cells with the wild type or mutant chimeric MLL/MLL2-AF9 fusions was performed to measure proliferation capacity in a methylcellulose colony assay. Colony forming ability and cell proliferation were measured along with changes in cellular morphology and target gene expression to determine the functional contributions of specific residues within the CXXC domain of the fusion proteins. The chimeric MLL/MLL2-AF9 fusion showed a significant reduction in proliferation capacity compared to MLL-AF9 along with decreased target gene expression and enhanced cellular differentiation. Interestingly, specific residues from MLL, when introduced into the chimeric MLL/MLL2-AF9 fusion protein, rescued the proliferation ability and target gene expression of the synthetic MLL/MLL2-AF9 fusion protein. The residues were found on both the DNA-binding surface and on the opposite, non-DNA-contacting surface of the domain. Subsequent *in vitro* binding studies revealed functional roles for these residues with altered DNA-binding affinities between MLL and synthetic MLL/MLL2 protein domains and without differential binding to known cofactor proteins. By comparing the residues of the MLL and MLL2 CXXC domains, critical amino acids were identified which may ultimately be targeted with small molecule therapies to improve the outcomes of these aggressive MLL leukemias.

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Lxrα Regulates Liver-Dependent and – Independent Pathways to Limit Atherosclerosis

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The liver X receptors LXRα and LXRβ are members of the nuclear hormone receptor superfamily of transcription factors that function as regulators of cholesterol homeostasis. In macrophages LXRs promote the efflux of internal cholesterol to high density lipoprotein particles (HDL) initiating the process of reverse cholesterol transport (RCT). We and others have demonstrated that treatment with LXR agonists reduces atherosclerosis in animal models of cardiovascular disease at least in part via up-regulation of macrophage cholesterol efflux. Genetic studies in LDL receptor (LDLR) knockout and ApoE knockout mice indicate that LXRα is responsible for the majority of the anti-atherogenic LXR activity. Additionally, LXRα functions in hematopoetic cells and in cells of other lineages to limit atherosclerosis. In the liver LXRα regulates the catabolism and excretion of cholesterol, classically considered the penultimate steps in the RCT process, leading to the net loss of cholesterol from the body. We now demonstrate that tissue-specific deletion of LXRα in the livers of LDLR knockout mice significantly increases atherosclerosis, indicating the liver as an important site of LXR-dependent anti-atherogenic activity. Our studies indicate that hepatic LXRa is important for regulating lipoprotein particle number, size, and function, and the elimination of this activity contributes to atherogenicity. Treatment of LXRα liver-specific knockout mice with an LXR agonist, however, still significantly decreases atherosclerosis suggesting that the ability to stimulate hepatic cholesterol catabolism and fecal excretion is not required for the anti-atherogenic activity of LXR agonists. Activation of liver LXR results in increased lipogenesis, which has stymied the development of LXR ligands for the treatment of cardiovascular disease; however, our studies indicate that activation of extra-hepatic LXR may still be of therapeutic potential.

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A Novel Behavioral Assay for Cold Nociception

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Background: Pain is a critical issue in patient care, and the NIH estimates that $200 billion/year is spent on pain-related issues. Patients report a variety of types of pain including mechanical pain, heat pain, and cold pain, all of which may have different molecular mechanisms. As the diverse mechanisms of nociception become better understood, more effective therapies targeted at specific mechanisms of pain can be developed to cater to individual patients' needs. In order to investigate the mechanisms of pain, simple and unambiguous animal models of pain behavior are necessary. The currently used behavioral assays for cold nociception are low throughput, stress the animals being tested, and utilize subjective measures of how the animals respond to a fixed stimulus instead of quantitatively measuring the cold response threshold. To address the deficiencies in the accepted behavioral assays of cold nociception, we have developed a novel approach to measuring cold pain in rodents. This simple method allows a consistent administration of a ramping cold stimulus to the hindpaw of mice until the paw is withdrawn. The behavior is quantified as the amount of time that the dry ice is applied to the paw before the paw is removed (withdrawal latency), and a longer latency implies that the mouse has a higher threshold for cold nociception or a higher tolerance for cold pain. The results show that this assay applies a consistent ramping cold stimulus to unrestrained animals, and that the response to this stimulus is consistent. The results also show that the withdrawal latency can evaluate morphine induced analgesia as well as hyperalgesia induced by injection of Complete Freund's Adjuvant or by the Spinal Nerve Ligation surgery. This approach offers an easily quantifiable way to assess the cold pain threshold of rodents without restraining the animals or relying on subjective approximations of animal responses, and should be useful for future studies of the molecular mechanisms of cold pain.

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The Chief Resident Aesthetic Surgery Clinic: A Safe Alternative for Patients

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Background: Providing comprehensive aesthetic surgery training is a major challenge in residency programs. Supervised chief resident aesthetic surgery clinics offer advantages for both trainees and patients: residents learn by hands-on care, and reduced fees offer patients affordable access to aesthetic procedures. In order to inform educators about the safety of the training model, an investigation of surgical outcomes from these clinics is needed. **Methods:** The authors performed a retrospective chart review of consecutive patients undergoing primary body contouring aesthetic surgeries through the Johns Hopkins chief resident aesthetic surgery clinic from July 2009 to July 2011. A single board certified attending surgeon supervised all operations. Breast procedures included augmentation with or without mastopexy, mastopexy alone, and reduction mammaplasty. Body contouring procedures included liposuction, abdominoplasty with or without liposuction, and belt lipectomy. The study included 115 patients who underwent 132 primary body contouring procedures (48 breast procedures, 84 body contouring procedures). Major complications and minor complication were identified. Additionally, we tabulated the incidence of hypertrophic scars requiring in-clinic revision. **Results:** Amongst 132 primary body contouring procedures, there were no emergent returns to the operating room. Two patients developed infection requiring hospitalization for intravenous antibiotics (major complication, 2.4%). The overall minor complication rate for breast and abdominal procedures was 6.3% and 21.4%, respectively; the overall revision rate was 2.1% and 14.3%, respectively. In-clinic scar revision accounted for 73% of all revisions. The incidence of seroma for breast and abdominal procedures was 1% and 10.2%, respectively; non-operative delayed wound healing, 1% and 11.9%, respectively; infection requiring oral antibiotics, 1% and 15.3%, respectively. There were no hematomas. **Conclusion:** In this study, we report complication rates comparable to those reported in the literature. As such, our chief resident clinic offers patients safe options with greater accessibility to aesthetic surgery compared to private aesthetic surgery practices. At Johns Hopkins, the chief resident aesthetic surgery clinic model continues to be an important component of our residency training program.

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Near-infrared Lymphography as a Minimally Invasive Modality for Imaging Lymphatic Reconstitution in a Rat Orthotopic Hind-Limb Transplantation Model

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Background: Vascularized composite allotransplantation (VCA) has become a prominent reconstructive option for patients suffering major tissue loss. Wider application is limited by the need for chronic immunosuppression to prevent graft rejection. Recent data suggest that the lymphatic system plays an important role in mediating allograft rejection. However, little is known about lymphatic reconstitution in VCA. The purpose of this study was to use a novel, minimally invasive imaging method, near-infrared (NIR) lymphography, to identify lymphatic reconstitution in rat orthotopic hind-limb transplants. **Methods:** Syngeneic (Lewis-Lewis) and allogeneic (Brown Norway-Lewis) rat orthotopic hind-limb transplants were performed with no immunosuppression. Histology confirmed indefinite and 7-10 day graft survival in syngeneic and allogeneic transplants, respectively. Animals were imaged pre- and post-operatively using indocyanine green (ICG) NIR lymphography. ICG was injected intradermally at multiple spots on the hind-limb. Images were collected for up to 14 days using a custom-built NIR imaging device. Images were reviewed to identify lymphatic reconstitution. **Results:** Prior to transplantation, all donor limbs exhibited a fine, organized lymphatic drainage

system. In both syngeneic and allogeneic transplants, ICG first crossed graft suture lines on post-operative (POD) 5; a fine network of vessels appeared in syngeneic transplants, but no vessels were seen in allogeneic transplants due to unresolved edema. Clinical signs of rejection also appeared on POD 5 in allogeneic animals. By POD 14, a dense, chaotic meshwork of vessels developed at the suture line of syngeneic transplants. **Conclusion:** NIR lymphography is a minimally invasive imaging modality that can be used for identifying reconstituted draining lymphatic vessels in a rat vascularized composite transplantation model. In allogeneic transplants, visualization of ICG in recipient tissues correlated with clinical signs of rejection. Further studies using NIR lymphography are needed to delineate this relationship.

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Retinoic Acid Contribution to Neural Crest Cells, Enteric Nervous System and Gastrointestinal Tract Development during Embryogenesis

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Hirschsprung's disease (HSCR) is a congenital absence of neurons in a portion of the intestinal tract, usually the distal bowel, due to a failure of neural crest cell (NCC) colonization or development. This congenital anomaly occurs in 1/5000 live births, and typically requires surgical resection of the aganglionic bowel. RDH10 is a retinol dehydrogenase enzyme required for the oxidation of vitamin A to its active form, retinoic acid (RA). Insufficient or excess RA can result in various congenital abnormalities and fetal death. The proposed studies investigate the role of vitamin A metabolism on neural crest cells and gastrointestinal tract development using an RDH10 knockout (*Rdh10^{trex}*) and conditional knockout (*Rdh10^{CKO}*) models. Specifically, this project will address the effects of retinoid deficiency in these retinoid deficient mice as well as investigate the cause and potential rescue of the HSCR phenotype in these mutant embryos. The results of these investigations will elucidate the importance of RA throughout embryonic development in the pathogenesis of HSCR. The long term goal of these research efforts will test the hypothesis that RDH10 has a significant impact on the RA synthesis necessary for organogenesis, specifically on NCC migration during formation of the enteric nervous system (ENS). Presumably, more severe RA deficiency during organogenesis will lead to more severe congenital abnormalities. We intend to characterize the development of the ENS in retinoid deficient embryos, determine the cellular mechanisms of the defective ENS in these mutants, and define precise temporal and spatial requirements for retinoid signaling for proper NCC colonization of the gut and complete ENS formation using *Rdh10^{CKO}* embryos. Additionally, to confirm the temporal requirement for RA signaling, we will rescue the retinoid deficient embryos via *in utero* retinoid supplementation. These novel models of Hirschsprung's will improve our understanding of retinoic acid contribution to intestinal development in the context of an HSCR model. This knowledge may lead to innovative non-surgical treatment approaches to reduce the morbidity and mortality of this common congenital disease.

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Preclinical Studies on a Novel Class Inhibitors of Lactate Dehydrogenase A (LDH-A) as Targeted Therapeutics against Cancer Cell Metabolism and the Warburg Effect

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We have recently reported a series of N-hydroxyindole (NHI) compounds as a novel class of lactate dehydrogenase A (LDH-A) inhibitors. The glycolysis enzyme LDH-A represents a novel and selective anti-cancer target since it is crucial in allowing cancers to maintain their highly-glycolytic metabolism (the Warburg effect), survive in hypoxic conditions, and maintain an acidic tumor microenvironment to help evade immune response. LDH-A overexpression has been correlated with poor prognosis and survival in a diverse array of human cancers, and emerging research presents compelling evidence for LDH-A inhibition as a tractable anti-cancer strategy. Here we perform further biological characterization on three promising NHI-class LDH-A inhibitors, 1j, its methyl ester 4j, and the glucose conjugate of 4j. We have found that 4j is more toxic to cancer cells, is more cell-permeable, and induces a more potent decrease in cancer cell lactate production compared to 1j. Glucose conjugation is an exciting anti-cancer strategy to increase targeting of drugs to cancerous vs. non-cancerous cells, capitalizing on the over-expression of GLUT transporters in many tumors. We have shown that glucose-4j competes with ^{13}C glucose for cellular entry, suggesting that it is a substrate for GLUT transporters and may preferentially be targeted to cancer cells in a living system, and that it is cleaved to 4j over time in cell culture. We have also demonstrated that glucose-4j is more cell-permeable, more toxic to cancer cells, and comparable in its ability to decrease cancer cell lactate production compared to 4j. We are interested in performing further biochemical characterization of this class of LDH-A inhibitors, including examining compound toxicity in normoxia vs. hypoxia and correlating cancer cell toxicity with LDH-A and GLUT expression levels. These cell culture characterization studies, along with ongoing animal studies using these compounds, will and push these compounds toward cancer clinical trials as well as broaden our understanding of LDH-A's role in cancer biology.

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The PI3K P110α-Rac1 Signaling Pathway is Essential for Kras-Induced Pancreatic Tumorigenesis

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Background: New drug targets are urgently needed to develop primary and adjuvant therapies for pancreatic ductal adenocarcinoma (PDA). Almost all PDAs are caused by mutations in the Kras gene. Pharmacological inhibition of Kras has so far proven unsuccessful. Fortunately, mutant Kras activates several

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molecules more easily targeted with pharmacological inhibitors, including the focus of our studies, phosphatidylinositol 3-kinase (PI3K). Our studies utilize the pancreas-specific Ptf1a-Cre;LSL-$Kras^{G12D}$ (abbreviated as KC) mouse model that faithfully reproduces human pancreatic tumorigenesis. Using the cre-lox system, we generated conditional knockout mice for PI3K catalytic isoforms p110α and p110β. We found that genetic ablation of p110α, but not p110β, in the mouse pancreas completely abrogated the development of pancreatic tumors in KC mice without causing detectable harmful effects. Furthermore, we generated a mouse strain expressing a constitutively active p110α mutant in the pancreas and found that activation of PI3K alone is sufficient to induce pancreatic tumor formation. Previous reports indicate that Kras can activate PI3K through direct binding to the Ras-binding domain of p110α. Surprisingly, replacement of pancreatic p110α with mutant p110α incapable of binding to Kras did not protect KC mice against tumorigenesis, indicating an indirect mechanism by which oncogenic Kras signals to p110α to induce pancreatic tumor formation. Interestingly, KC pancreata exhibited markedly elevated p110α expression, suggesting an alternative mechanism by which Kras activates PI3K in our model. We also investigated signaling pathways downstream of PI3K in KC mice. Unexpectedly, Akt, a known downstream effector of PI3K, did not show increased activation in the KC pancreas, and loss of p110α did not decrease its activation level. Upon exploring alternate PI3K effectors, we found that the KC pancreas exhibited a marked increase in activation of Rac1, a small G protein involved in actin cytoskeleton rearrangement. More importantly, ablation of p110α, but not p110β, completely blocked this Rac1 activation, a finding consistent with a previous study showing that ablation of Rac1 blocks oncogenic Kras-induced pancreatic tumorigenesis. In summary, we found that Kras requires and activates the p110α-Rac1 pathway to induce pancreatic tumorigenesis through an indirect mechanism and not through direct binding to p110α. Further studies are needed to determine if pancreatic tumor maintenance requires the continual presence of p110α signaling in order to assess the viability of targeting this enzyme in treating PDA.

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Sinus Hypoplasia Precedes Sinus Infection in a Porcine Model of Cystic Fibrosis

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Chronic sinusitis is nearly universal in humans with cystic fibrosis (CF) and is accompanied by sinus hypoplasia (small sinuses). However, whether impaired sinus development is a primary feature of loss of the cystic fibrosis transmembrane conductance regulator (CFTR) or a secondary consequence of chronic infection remains unknown. To study the early pathogenesis of sinus disease in CF, we examined sinus development in a porcine CF model. Porcine sinus epithelia expressed CFTR and exhibited transepithelial anion transport. Disruption of the *CFTR* gene eliminated both. Sinuses of newborn CF pigs were not infected and showed no evidence of inflammation, yet were hypoplastic at birth. Older CF pigs spontaneously developed sinus disease similar to that seen in humans with CF. These results define a role for CFTR in sinus development and suggest the potential of the CF pig as a genetic model of CF-sinus disease in which to test therapeutic strategies to minimize sinus-related CF morbidity.

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Mechanism of DAF-16-Dependent Lifespan Extension by SGK-1

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The FOXO/DAF-16 family of transcription factors has been implicated in aging and age-related diseases in species as diverse as invertebrates and mammals. In C. elegans, loss-of-function mutations in the conserved insulin-like signaling pathway result in upregulation of the transcriptional activity of C. elegans FOXO homolog DAF-16, which is sufficient to extend adult lifespan severalfold and increase resistance to multiple forms of stress. The best-characterized function of insulin-like signaling is to sequester DAF-16 in the cytoplasm and inhibit its activity via phosphorylation by the serine-threonine kinases AKT-1 and AKT-2. Similarly, the highly related kinase SGK-1 acting in parallel to AKT1/2 can also phosphorylate DAF-16, and mammalian cell culture data suggests it plays a similar role as AKT1/2. However, while AKT1/2 are well established suppressors of lifespan, recent experiments using both loss- and gain-of-function mutations show that SGK-1 increase lifespan in C. elegans, indicating that SGK-1 may be activating DAF-16 transcriptional activity through an unknown mechanism. Two possibilities exist: 1) SGK-1 is controlling a novel parallel pathway that regulates DAF-16, or 2) the preference of AKT1/2 and SGK-1 for different but overlapping DAF-16 phosphorylation sites dictates unique effects on DAF-16 activity. Here, we present our work testing the hypothesis that SGK-1 activates DAF-16 directly through phosphorylation of one of these sites using both genetic and biochemical approaches. We also characterize the stress resistance profiles of sgk-1 mutants to elucidate the downstream effectors of DAF-16 activation by SGK-1. Because the mechanisms controlling FOXO/DAF-16 activity are highly conserved, insights into SGK-1's role in C. elegans longevity can be applied to human diseases involving FOXO including cancer, diabetes, and osteoporosis.

30 (Cancelled)

Association between *CLU* Polymorphisms and Plasma Lipid Levels in Sporadic Alzheimer's Disease

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The genetic factors have been found to play critical roles in Alzheimer's disease (AD) pathogenesis. Mutations in *APP, PSEN1* and *PSEN2* genes are confirmed to be causative for family AD.

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For sporadic AD, it is suggested to be a multifactorial complex disease and caused by different genetic risk factors. Recently, *CLU* encoding Clusterin, is simultaneously reported to be associated with sporadic AD by Genome-wide association studies (GWAs) based on Caucasian populations. Although successful follow-up study for *CLU* and sporadic AD susceptibility has been performed in our independent Chinese population, we further analyze those significant single nucleated polymorphisms (SNPs) and plasma lipid levels based on 689 participants. Subjects including 400 AD and 289 controls are recruited from the Memory Clinic, Queen Mary Hospital and community elderly social centers, respectively in Hong Kong. Cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C) as well as low-density lipoprotein cholesterol (LDL-C) levels are measured to evaluate subjects' plasma lipid levels. By multivariate logistic analysis, after adjustment for age, sex and *APOE*ε4 status, two SNPs (rs2279590 and rs11136000) are found to be significant correlations with lipid levels. Both A allele of rs2279590 and T allele of rs11136000 are positive correlations with HDL-C levels, while negative correlations with triglyceride levels. This is a first study that exhibition the associations between *CLU* polymorphisms and plasma lipid levels in sporadic AD. Since *in vivo* study has reported the association between soluble Aβ and HDL particles, findings from our present study afford additional evidence of *CLU* susceptibility in sporadic AD development.

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Epithelial STAT3-Induced Antimicrobial Protein, Reg3γ is Required for Host Defense against MRSA Pneumonia in Mice

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Rationale: Incidence of community-associated Methicillin-resistant *Staphylococcus aureus* (CA-MRSA) pneumonia has increased and is associated with bronchiectasis, compromised lung function, and high mortality rates. The mechanisms of host defense against this pathogen are not fully characterized. However, patients with Hyper IgE Syndrome (HIES), resulting from mutations in signal transducer and activator of transcription 3 (STAT3), are susceptible to recurrent SA skin and pulmonary infections. **Methods:** USA300, the most prevalent pneumonia-causing strain in United States, was used to identify the necessary immune cells and signaling pathways for host defense. USA300 ($2x10^7$ CFU) was oropharyngeally administered to C57BL/6 (WT) and various knockout mice: TLR2$^{-/-}$, IL-1R1$^{-/-}$, IL-17RA$^{-/-}$, NLRP3$^{-/-}$, and Rag2γc$^{-/-}$. Bacterial burden, gene expression, and protein levels were determined in the lung 20 hours post-infection. **Results:** Although, Rag2γc$^{-/-}$, IL-17RA$^{-/-}$, and NLRP3$^{-/-}$ mice clear SA as well as WT mice, IL-1R1$^{-/-}$ and TLR2$^{-/-}$ mice had increased bacterial burden. USA300 infection leads to increased GP130 ligands; IL-6 binding to GP130 and triggering STAT3 phosphorylation is the best-characterized activation of STAT3. Induction of IL-6 also occurred in Rag2γc$^{-/-}$ mice demonstrating that B, T, NK, and NK T cells are dispensable for this response. GP130 or STAT3 inhibition results in increased bacterial burden. IHC shows STAT3 activation in lung epithelium. IL-6 induces STAT3 signaling and Reg3γ production by mouse lung epithelial (MLE12) cells. Furthermore, STAT3-dependent Reg3γ limits USA300 growth *in vitro*. **Conclusion:** TLR2, IL-1R1, and STAT3 signaling are necessary for host defense against USA300 pneumonia. Although, HIES patients have mutations in STAT3 resulting in decreased Th17 cells, Th17 cells do not appear to be necessary for the pulmonary clearance of USA300, because Rag2γc$^{-/-}$ mice, without B, T, NK, or NK T cells, clear USA300. Recurrent SA pneumonias in these patients are probably due to impairment of STAT3 signaling in innate immune and epithelial cells. Reg3γ has antimicrobial activity against USA300, which may be the basis for new therapies for CA-MRSA pneumonia. **Funding:** 5R37HL079142, 5T32AA007577-09, 1F31AA021601

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Inhibition of MTOR Alters Autophagic Flux in Podocytes and Results in Proteinuria

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Mammalian target of rapamycin (MTOR) inhibitors belong to a family of drugs with potent immunosuppressive, anti-angiogenic and anti-proliferative properties. Although they are approved for the treatment of a number of renal diseases, the use of MTOR inhibitors has been associated with a significant incidence of de novo or worsening proteinuria. Here we explore the mechanism of proteinuria induced by MTOR inhibition through the generation and characterization of mice carrying a podocyte-selective knockout (*Mtor* pod-KO) of the *Mtor* gene resulting in a loss of both Mtor complex 1 and 2 functions. Normal glomerular development in *Mtor* pod-KO mice suggests that Mtor is dispensable in developing podocytes. However, *Mtor* pod-KO mice develop nephrotic range proteinuria by 11 days of age, and progress to end-stage renal failure by 3 weeks. *In vitro*, Human podocytes treated with the mTOR inhibitor rapamycin accumulate autophagoloysomes, damaged mitochondria and increased levels of reactive oxygen species. *In vivo*, podocytes of *Mtor* pod-KO mice exhibit an accumulation of the autophagosome marker LC3 (rat microtubule-associated protein 1 light chain 3), autophagosomes, autophagolysosomal vesicles and damaged mitochondria. Taken together, our results suggest that disruption of the autophagic pathway may result in mitochondrial dysfunction and play a role in the pathogenesis of proteinuria in patients treated with mTOR inhibitors.

Poster Abstracts

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Deep Brain Stimulation Entrains and Depresses Local Neuronal Firing in Human Globus Pallidus Internus

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Deep Brain Stimulation (DBS) in the internal segment of the globus pallidus (GPi) relieves the symptoms of Parkinson's Disease through a yet unknown mechanism of action. Since high-frequency electrical stimulation works in many of the same sites as ablation or lesion, DBS was originally proposed to act through reversible silencing of local neuronal activity. This hypothesis was supported by the observations that GPi neurons have increased firing rates in Parkinson's Disease, and that microelectrode stimulation of GPi in small animal models produces complete inhibition of firing with concomitant relief of bradykinesia. However, recent evidence in humans and primates indicates that the therapeutic mechanism of DBS is not through uniform inhibition of neuronal activity. To address the question of how therapeutic stimulation changes local neuronal firing in the human brain, we studied the effects of stimulation on local neuronal firing in the GPi of awake patients receiving bilateral DBS implants. Eleven patients receiving treatment for Parkinson's Disease consented to participate in recordings during the implantation of DBS leads into GPi (IRB #00006169). In non-anesthetized patients, a recording microelectrode was advanced concurrently with a stereotaxically guided DBS electrode into the GPi, as verified by intraoperative X-ray. Single neurons were isolated with the microelectrode, followed by stimulation through the DBS electrode. Of the recordings, 45 neurons and 186 stimulation trials were of sufficient quality for spike-sorting and further analysis. The intra-operative stimulation parameters (1-8V, 88-180Hz, 0.1ms pulses) were verified post-operatively as similar to the optimal DBS settings for long-term symptom relief. Stimulation in the GPi did not eliminate local neuronal firing, but decreased the net activity in a voltage dependent fashion. Control stimulation outside of the GPi did not significantly change the firing rate. Thirty-three (33) of 45 neurons also displayed phase-locked firing relative to the stimulus, with a period of increased probability of firing 2-5ms post-pulse followed by a 2-3ms period of decreased probability of firing. A Poisson surprise analysis showed no change in burst firing between pre- and post-stimulation. These data show that DBS in human GPi does not produce total neuronal inhibition. Rather, DBS caused a decrease in overall firing rate, with a time-locked, biphasic pattern of excitation and inhibition relative to the stimulus. These findings are consistent with computational models and with recordings from primate GPi showing that stimulation of the GPi inhibits pathologically increased firing rates through partial inhibition and entrainment.

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Is Repeat Testing for *Clostridium Difficile* Infection Using Either Toxin EIA or Real-Time PCR for Toxin B Gene of Any Diagnostic Value?

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Background & Aims: The standard diagnostic protocol for suspected *Clostridium difficile* infection (CDI) has been to send 3 successive stool samples to increase the sensitivity of the commercially available enzyme immunoassay (EIA) tests. The aim of the study is to determine the value of such a practice and to compare it with polymerase chain reaction (PCR) which is known to have a better sensitivity and specificity. **Methods**: A retrospective cohort study was designed using a database of all stool EIAs and PCR that were performed on hospitalized patients between January 2005 and May 2011. The results of the first three tests were taken into consideration for each episode of diarrhea. The data was also analyzed for the test's sensitivity based on the first three tests. Also transition probabilities were calculated based on repeat testing results. **Results**: A total of 56,583 and 6,503 patients over a course of five and a half years were tested for CDI using EIA or PCR respectively. Of those, 5218 (9.2%; CI 9.0-9.5%) and 860 (13.2%; CI 12.4-14.1%) patients were diagnosed positive with EIA and PCR respectively. The first stool sample tested was positive in 90% and 94% of patients with EIA and PCR respectively. If the first stool EIA or PCR test was negative, the probability that the second stool test will be positive is 2.7% (95% CI 2.5–3.0) and 3.9% (95% CI 3.0-5.1). **Conclusion**: Most patients with CDI were diagnosed on the first EIA (90%) or PCR (94%) test itself. The diagnostic yield of repeat stool testing is low with EIA or PCR. Per present guidelines testing three stool specimens is not recommended routinely (SHEA position paper). The increase in the rate of CDI with PCR testing needs to be investigated further to confirm if it is true positivity or asymptomatic carriage (false positives). We suggest that there may be a need to switch to PCR which has a better sensitivity and does not require repeat stool testing.

35 (Cancelled)

Characterization of Skeletal Muscle-Specific Overexpression of Mitochondrial Transcription Factor A (Tfam) in Mouse

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There is continuing controversy on whether skeletal muscle mitochondrial capacity impacts on insulin sensitivity. Thus, we created a transgenic mouse model with skeletal muscle specific (Muscle creatine kinase promotor) overexpression of human mitochondrial DNA binding protein transcription factor A (Tfam), known to promote mitochondrial biogenesis and mtDNA replication. Male Tfam and Wildtype littermate mice were fed

a custom high fat (HF) or control diet from 4 weeks to 16 weeks of age. The Tfam animals, with significantly higher mtDNA copy number, are smaller than the WT on both diets ($p<0.05$). Tfam HF mice demonstrated higher day and night O2 consumption in comparison with WT by 24 hour indirect calorimetry. Body composition by Echo MRI demonstrated that the Tfam mice have fat mass comparable to WT, but significantly lower lean mass ($p<0.05$). Most notably the muscle wet weight at sacrifice was significantly reduced for gastrocnemius, tibialis anterior, plantaris, quadriceps and EDL in Tfam HF and Tfam C compared to WT (with and without correcting for total body mass). The muscles are also uniformly more red in color (n=4-7 per group) as reported in PGC1-alpha overexpressing transgenic mice. Tfam animals exhibited significantly reduced run times on endurance testing compared to WT, irrespective of diet, (Tfam 96 minutes vs WT 143 minutes, $p<0.05$) without measureable differences in VO2 max. Thus, skeletal muscle specific overexpression of human Tfam in mouse results in significantly reduced muscle mass and a high fat diet challenge resulted in similar fat gain with smaller muscle mass gain. Smaller body mass on HF feeding in Tfam mice seems to be related to lower muscle mass rather than fat stores in Tfam HF mice.

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Effect of CYP2B6 Variants on Chlorpyrifos Metabolism: Implications for Human Risk

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Introduction: Chlorpyrifos (CPF), a widely used organophosphorus (OP) pesticide, must be bioactivated by cytochrome P450s (CYPs) to chlorpyrifos oxon (CPF-O), a potent cholinesterase inhibitor. CYP2B6 has the highest reported intrinsic clearance (CL_{int}) for bioactivation of CPF, which suggests an important role in chronic occupational CPF exposures. Furthermore, CYP2B6 is a highly polymorphic enzyme and variants of this enzyme may impact human susceptibility to this OP. In this study, human CYP2B6*1, *4, *5, *6, *7, and *18 were over-expressed in mammalian COS-1 cells to assess the impact of CYP2B6 variants on the K_m and V_{max} for bioactivation of CPF. Human liver microsomes (HLMs) genotyped for the most common variant CYP2B6*6 were also used. *Methods:* Cell lysates were incubated with CPF (0-100μM) and the production of CPF-O was measured via HPLC analysis. CYP2B6 content was determined by western blot. In addition, human liver microsomes (N=22) genotyped for CYP2B6*1 and CYP2B6*6 were assayed for ability to metabolize CPF at 10μM and at 0.5μM, concentrations straddling the predicted K_m. **Results:** CYP2B6*18 had neither detectable activity toward CPF nor detectable protein. CYP2B6*4 had a significantly higher V_{max} and CL_{int} than CYP2B6*1 enzyme (414 versus 26.4 nL/min/nmol CYP2B6, respectively), while the CL_{int} values for CYP2B6*5 and *7 (229 and 207 nL/min/nmol CYP2B6, respectively) were also significantly greater than wild-type. For HLM specimens, homozygous CYP2B6*6 had both reduced protein expression and CPF-O metabolite formation. This difference was not significant when the data were expressed as pmol/min/nmol CYP2B6 instead of per mg total protein. **Conclusions***:* Together, these data support the conclusion that variants of CYP2B6 may have altered capacity to bioactivate CPF and affect individual susceptibility by altering hepatic expression of CYP2B6 protein (CYP2B6*6) and/or kinetic parameters for CPF-O formation (CYP2B6*4, *5, & *7). The kinetic data generated here may be used to assess the impact of CYP2B6 genotype on current human risk assessment efforts for CPF which currently rely on rat kinetic data and may under represent human variability. Together with hepatic expression data for each variant, this knowledge will help identify and protect susceptible worker populations. (Funding: NIH R01 ES016308 and EPA STAR grant R833454)

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A Bacteriophage Virus-Like Particle Display Platform for Conformational Peptide Epitope Identification Using Affinity-Selection

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Filamentous phages are now the most widely used vehicles for phage display and provide efficient means for epitope identification. However, the peptides they display are not very immunogenic because they fail to present foreign epitopes at the high densities required for efficient B-cell activation. Meanwhile, systems based on virus-like particles (VLPs) permit the engineered high-density display of short peptide epitopes. We have developed a peptide display platform based on VLPs of the RNA bacteriophages MS2 and PP7 that combines the high immunogenicity of VLP display with affinity selection capabilities. Specific or random peptides can be displayed on the VLP surface by genetically inserting a sequence into an exposed loop of the viral coat protein. Displayed peptides can then be selected upon specific antibody screening, and the VLPs used directly as immunogens. Preserved in this system is the ability of the VLP to package the entire coat protein-encoding mRNA including the insertion, linking VLP genotype and phenotype. We have previously shown that with the both RNA bacteriophage MS2 and PP7, >95% of insertions are compatible with VLP assembly, using random peptide sequences of either 6, 8, or 10 amino acids. As a proof-of-principle experiment, we have used this system to identify linear epitopes for several previously characterized monoclonal antibody targets (the Flag peptide and a linear epitope from anthrax protective antigen) and showed that the VLPs thus obtained elicit antibodies in mice whose activities mimic those of the selecting antibodies. We have recently investigated the ability of this platform to identify mimotopes of conformational epitopes, using the anti-AMA1 (a Plasmodium falciparum conserved surface antigen) 4G2dc1 monoclonal antibody to screen PP7 and MS2 VLP-displayed random peptide libraries. Using these VLP screening platforms, we have identified specific peptides that may be acting as mimotopes of conformational epitopes in the context of the VLP platform, and may be amenable to VLP-based vaccination strategies. Using this strategy, we have identified a VLP-peptide selectant that elicits an AMAI-specific antibody response.

Poster Abstracts

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Astrocyte Senescence as a Component of Age-Related Neurodegeneration

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Astrocytes are a highly abundant population of glial cells that perform a myriad of complex functions in the central nervous system (CNS). In order to support neuronal homeostasis, astrocytes regulate the contents of the synaptic cleft, contribute to CNS metabolism, and maintain blood brain barrier (BBB) integrity. An alteration in these activities may contribute to age and disease-related neuropathology and our laboratory is exploring the hypothesis that cellular senescence in astrocytes is a causative event in age-related neuropathology. We have shown that astrocytes are capable of triggering a senescent growth arrest *in vitro* and these cells are highly sensitive to oxidative stress. We examined astrocytes for biomarkers of senescence in postmortem tissues from patients of differing ages and those with Alzheimer's disease (AD). We found an increase the percentage of astrocytes that are glial fibrillary acidic protein (GFAP)/ $p16^{INK4a}$-positive by immunofluorescence in the frontal cortices of aged and AD patients without a concomitant increase in the cerebellum, which is an area of the brain typically unaffected by AD pathology. It is known that senescent cells are functionally altered and secrete cytokines and proteases that contribute to age-related declines in organ function. While this senescence-associated secretory phenotype (SASP) has been characterized in fibroblasts, the mechanism involved in the regulation of the SASP in astrocytes is not well understood. We profiled the secretion pattern of senescent astrocytes *in vitro* and found an increase in a sub-set of proinflammatory cytokines suggesting that astrocytes produce a senescence-associated secretory phenotype (SASP). The recognition of the SASP in senescent astrocytes represents a novel area for potential interventions in the treatment of age- and disease-related neuroinflammation that will either ameliorate the effect of the SASP in the microenvironment or enhance the clearance of the senescent astrocytes via the immune system.

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Modulating Cancer Progression with Zymogen Protein C in a Murine Model

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Recent studies have demonstrated the ability of activated protein C (APC) to control cancer progression in a murine model of melanoma, though the mechanism has yet to be fully elucidated. Data has suggested that the cytoprotective effect, mediated via the protease-activated receptor, PAR-1, of APC is necessary for protection. To explore this, we generated murine (m) APC variants and tested their ability to prevent metastasis. AAV8-mediated gene therapy was used to express the variants to ensure continuous expression at constant levels, after which $2.5x10^5$ B16F10 murine melanoma cells were delivered intravenously. Lung tumor rates of variant-treated animals were compared to age and gender matched saline-injected groups. Mice expressing wildtype (WT) mAPC at levels of 7.3 ± 1.5 ng/ml or lower did not differ from controls (mAPC levels <3 ng/ml), while WT mAPC levels of 25.6 ± 4.8 ng/ml to 118 ±6 ng/ml did reduce the number of lung metastasis compared to saline injected mice ($p<0.05$). Mice expressing the mAPC-5A mutant, which has intact cytoprotective activity but ~10-fold less anticoagulant activity, at levels of 15.2 ± 3.2 ng/ml to 80.4 ± 4.7 ng/ml exhibited rates of lung metastasis similar to controls. In contrast, expression of the mAPC-L38D mutant, which has ~2-fold less anticoagulant activity due to impaired protein-S interaction, at levels of 87± 25 ng/ml was effective in preventing tumor metastasis ($p <0.004$). To further investigate the role of the anticoagulant pathway in the APC anti-metastasis effect, we tested the ability of zymogen protein C (PC) to control tumor progression. mPC levels showed dose-dependent increases up to 8-fold of normal, with no increases in mAPC levels compared to saline-injected controls. Remarkably, mice expressing WT mPC had significantly reduced tumor metastases compared to controls (99% reduction; $p<0.0001$). mPC protected against metastasis even in PAR-1 null mice ($p<0.01$). WT mAPC, and to some extent mAPC-L38D, led to prolongation of the aPTT and increased blood loss after tail clip in a dose-dependent manner, while mAPC-5A and mPC did not. Notably, the half-life of PC is >20-fold longer than that of APC, making it a more attractive pharmacological agent. These findings support a novel, important role for zymogen PC in modulating tumor progression with minimal risk of bleeding.

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Long-Term Developmental and Educational Attainment of a Cohort of Children with Isolated Fetal Ventriculomegaly (IVM)

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Isolated ventriculomegaly (IVM) is defined as an increase of ventricular atrial width of ≥ 10 cm without evidence of congenital infection, structural or chromosomal abnormality. The reported incidence of neurodevelopmental impairment is 0.0-19.0% but there are no long-term follow-up data. The aim to evaluate the educational and main neurodevelopmental outcomes in children with antenatal IVM. A postal questionnaire defining neurodevelopmental, medical and educational outcome was sent to families of eligible cases from St. Mary's Fetal Management Unit. Outcome was related to the degree of IVM (mild or moderate/severe; resolving/regressive, stable or progressive). Results showed that 167 foetuses with IVM were identified (mean ventricular size:13.2±4.3 cm; mean-23.5 weeks); 49 of these aborted, 44 therapeutically. We located 84 families and 39 (46.9%) returned questionnaires and 9 (28.2%) underwent a standardized clinical assessment: 25 males, 14 females; mean age 12.6±2.8 years (range:9.0-19.2) and mean maternal age 29.3±5.4 (range:15.9-38.8). About 64% (95% CI, 48.4-77.3%) with IVM required additional

educational support and 25.6% (95% CI, 14.6-41.1%) attended a special needs school. Delayed walking (mean 16.3±7.1 months) was seen in 41% (95% CI, 27.1-56.6%). Epilepsy was reported in 12.8% (95% CI, 5.6-26.7%). A significant association was found between moderate and severe IVM additional educational support (P=0.004) and delayed walking (P=0.006). In conclusion, children with IVM are more likely to require educational support in the form of speech therapy, additional help at class and likely to attend in special needs schools compared to the general population. The severity of IVM in pregnancy renders children more likely to receive educational support at school, whereas IVM that is not resolving or regressing at serial antenatal ultrasonography is associated with a higher risk of motor-perceptual deficits. This is the first study providing prognostic information for families, paediatricians and educational psychologists as to the developmental and educational needs of older children diagnosed with IVM.

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Do Proteins Capable of Causing Spongiform Neurodegeneration Induce Aggresomes *In Vitro* and *In Vivo* as an Indicator of Their Neurotoxic Mechanisms?

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Objective: To explore the generality of aggresome formation in neurodegeneration, we investigated whether aggresomes arise in two spongiform neurodegeneration models, in which vacuolation is induced by neural stem cell (NSC) dissemination of puromycin-N-acetyltransferase (PAC) or retroviral envelope (Env) in the developing central nervous system (CNS). **Background:** Several human neurodegenerative diseases are characterized by the presence of protein deposits called aggresomes. A previous report indicated that expression of PAC in certain cultured cells causes protein aggregation. Additionally, *in vivo* studies from our laboratory suggest that astrocytic expression of PAC induces a spongiosis similar to that induced by Env. The present study collates these findings to understand whether aggresomes are an indicator of protein toxicity *in vitro* and *in vivo*. **Design/Methods:** Aggresome markers were used to assay intracellar aggregates in HeLa and glial cells transfected/transduced with vectors containing either Env or PAC and green fluorescent protein (GFP), a cellular identification marker. *In situ* studies were executed by immunostaining for aggresomes in neonatal mice transplanted with NSCs disseminating PAC or Env. Pathologically refractive NSCs transduced with PAC displaying surface Env served as controls. **Results:** No aggresome formation was detected in response to neurotoxic protein transfection/transduction; however, these proteins did induce an unexpected down-regulation in GFP protein expression. Moreover, brains engrafted with PAC-transduced NSCs precipitated focal spongiform neurodegeneration, thereby overcoming a previous restriction noted with Env. These NSCs differentiated into oligodendrocytes suggesting that PAC influenced their *in vivo* fate and capacity for inducing spongiosis. **Conclusions:** The present studies indicate that while spongiogenic proteins do not universally induce aggresome formation, they can alter cellular physiology by influencing endogenous protein expression and modifying cellular differentiation programs that result in neuronal dysregulation. Whether these activities are broadly applicable to other neurodegenerative diseases mediated by abnormal proteins remains to be investigated.

Study Supported By: This study was supported by the American Academy of Neurology.

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Metabolo-Genetic Profiling of Her2/Neu Mammary Tumor Recurrence

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Breast cancer is the most commonly diagnosed malignancy in women and is the second leading cause of cancer-related death in women in the U.S. Among women with breast cancer, tumor recurrence represents the principal cause of mortality. Nevertheless, little is known about the molecular mechanisms underlying how breast cancer cells survive therapy and ultimately recur. In particular, while dysregulated metabolism has long been recognized as a key feature of cancer development the metabolic changes accompanying cancer recurrence are largely unexplored. To address this gap, our laboratory has developed a series of inducible bitransgenic mouse models that accurately recapitulate human breast cancer progression, including primary tumor development, minimal residual disease, tumor dormancy and recurrence. The goal of this study was to determine whether metabolic differences exist between primary and recurrent mammary tumors arising in genetically engineered mouse models using ^{1}H magnetic resonance spectroscopy (MRS) and to correlate those differences with changes in the mRNA expression levels of enzymes involved in the production or consumption of those metabolites. These metabolo-genetic profiling experiments revealed a distinct metabolic signature for tumor recurrence. Compared to primary tumors, recurrent tumors displayed higher levels of lactate ($p=0.009$) and glycine ($p=0.001$), lower levels of succinate ($p=0.009$) and phosphocholine (PC) ($p=0.013$), and a higher glutamate:glutamine ratio (glu/gln) ($p<0.001$). These changes in steady-state levels of succinate, glu/gln, phosophocholine, glycine and lactate were accompanied by up-regulation of *Sdhb* and *Gls*, as well as down-regulation of *Chka*, *Gldc* and *Ldhb* in recurrent tumors that could potentially explain the observed shift in metabolic profile during recurrence. Pearson analysis confirmed a significant correlation between metabolite levels and expression of the corresponding gene regulating that metabolite ($r>0.49$, $p<0.05$). Together, our results indicate that primary and recurrent tumors differ with respect to their metabolic profiles. Investigating the underlying molecular determinants of these metabolic differences could enable the identification of new therapeutic targets and prognostic markers for breast cancer by improving our understanding of the molecular pathways involved in breast cancer recurrence.

Poster Abstracts

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The Trafficking and Processing of the Amyloid Precursor in Neurons

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APP metabolism leads to accumulation of the Aβ peptide within the brains of Alzheimer's disease patients. However, most of APP is processed through a non-amyloidogenic pathway to produce the p3 peptide, instead of Aβ. Regulation of APP processing within neurons is unclear. Our hypothesis is that the amyloidogenic processing of APP is regulated by polarized sorting of APP within neuronal processes. To test this hypothesis, I have created APP constructs that are selectively polarized to either the dendritic or axonal membranes of primary hippocampal neurons in culture. At 16 days *in vitro*, I have quantified the level of polarization by immunofluorescence and have shown the amount of Aβ that was secreted by Western blot. The different Abeta species were also analyzed by MALDI-TOF-mass spectrometry.

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Regulation of Endothelial Cells and VEGF Activity

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Endothelial cell dysfunction is a major contributing factor to cardiovascular disease. Vascular endothelial growth factor (VEGF) interacts with endothelial cell receptors VEGFR1 and VEGFR2 to stimulate endothelial cell proliferation, permeability, and migration. VEGFR2 is traditionally considered the active receptor mediating cellular responses. Due to its role in stimulating the growth of new blood vessels and maintaining viability of existing endothelial cell layers, significant attention has been aimed at understanding how to control VEGF interactions. We propose that mechanical and biochemical factors work together to govern the endothelial cell response to VEGF in a manner that is specific to each individual system. The mechanical environment surrounding endothelial cells varies significantly across different organ systems and disease states, suggesting that substrate mechanics may be a principal regulator of endothelial cell function. The goal of the present study was to explore how the mechanical state of the extracellular matrix modulates endothelial cell response to VEGF. As the substrate stiffens we predict that matrix bound VEGF will have more opportunities to interact with VEGFR2 through fibronectin and integrin associations. To study these interactions, we developed fibronectin functionalized polyacrylamide gels of defined stiffness. When aortic endothelial cells were cultured on these gels, VEGF mRNA levels did not change with stiffness, however, the levels of VEGFR2 and β1 integrin both increased on stiff substrates (135 kPa) compared to soft substrates (25 kPa) by ~2-fold. Increased VEGF stimulation has been shown to increase VEGF receptor levels, thus, it is possible that increased VEGF signaling on stiff matrices may occur even without increased VEGF. Toward this end, additional studies indicated that VEGF binding to fibronectin within the gels was increased in stiff compared to soft matrices. Hence, more fibronectin may be localized to the stiff fibronectin matrix for presentation and stimulation of endothelial cells leading to upregulation of VEGFR2 and increased angiogenic activity. Future studies will explore endothelial cell proliferation, migration, and signaling to determine if angiogenic activity is mediated by stiffness. Confocal microscopy will be utilized to explore possible interactions between focal adhesions and VEGF-VEGFR2 complexes. A better understanding of how the mechanical properties of the ECM control endothelial signaling could help identify new targets and approaches for cardiovascular disease treatment, and may provide a basis for the design of methods for directed tissue regeneration.

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Diagnostic Value of Repeat Stool Testing in Inflammatory Bowel Disease Patients Suspected with Clostridium Difficile Infection

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Background: The incidence and severity of *Clostridium difficile* infection (CDI) is increasing in patients with inflammatory bowel disease (IBD). The toxin enzyme immunoassay (EIA) or real time polymerase chain reactions (PCR) are being used to diagnose CDI. One earlier study on patients with IBD suggested that more than 1 stool sample be tested to increase diagnostic yield in these patients. We investigated whether repeat stool testing improves the diagnostic accuracy for CDI in IBD and non-IBD patients.
Methods: We performed retrospective data analysis from January 2005 to May 2011 on 63,086 hospitalized patients who were tested for CDI using EIA or PCR. Of these, 2579 IBD patients tested using EIA were analyzed separately. Transition probabilities were calculated based on results from repeated tests. **Results:** Of the 63,086 inpatients tested, 56,583 were tested using EIA and 6,503 were tested using PCR. The first stool sample tested was positive in 90% and 94% of patients with EIA and PCR respectively. Of the 2579 IBD patients tested using EIA, 101 were diagnosed with CDI. The first stool sample tested was positive in 81% of patients. Successive second and third stool samples yielded additional 14% and 5% CDI positive IBD patients. **Conclusions**: About one in five IBD patients with CDI yielded positive result with repeat testing after an initial negative test result with EIA. There are minimal diagnostic gains of repeat testing by EIA or PCR for non-IBD inpatients. Considering the short and long-term impact of CDI on IBD disease course, we recommend that three successive stool samples be tested for *C. difficile* toxin when using EIA to increase diagnostic yield in IBD patients with suspected CDI.

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Neural Coding of Corrective Movements in Motor Cortex

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It has been proposed that during a target switch reaching paradigm, the neural activity from motor cortex for the movement from starting point A to the original target B (AB) is replaced after the target switch with the neural activity for the motion from A to the new target C (AC). It is also known that the velocity profile for the target switch resembles the vectorial summation of the original AB velocity profile with that of the movement from original target B to the new target C (BC). Therefore, we considered the original as well as two competing hypotheses for neural coding of a target switch from B to C: (1) that coding for AB is replaced with AC, (2) that coding for AB is replaced with BC, and (3) that coding reflects the vectorial summation of the kinematics AB+BC. We recorded neural activity from primary motor and/or dorsal premotor from a total of three rhesus macaques (two subjects per cortical area) performing elbow flexion/extension movements between five targets. These control trials were pseudorandomly interleaved with target switch trials, where the target jumped to a new location after the start of the original movement. This required a second, corrective movement to be planned and executed in order to successfully acquire the new target. We found that hypothesis (2) - AB replaced with BC - explained the neural data significantly better than the two alternatives.

We interpret this as evidence for "intermittent" motor control, whereby movement is constructed from a basis set of movement primitives or templates, because the planning of the corrective motion began with the endpoint of the original motion. Additionally, the neural activity is not simply a linear function of the kinematics, as predicted by hypothesis (3). Instead, neurons seem to exhibit a dramatic state change when the corrective motion is planned. This is potentially relevant for the design of brain machine interfaces, whose decoding algorithms often assume that neuronal activity is a linear function of the kinematics.

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Histone Deacetylase Inhibition-Mediated Cytotoxicity of Oligodendrocyte Precursor Cells

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Histone deacetylase complex (HDAC) inhibition mediated by small molecule HDAC inhibitors (HDACi) is toxic to transformed cell lines but is also beneficial in a variety of neurological disease models. Recent studies indicate post-translational deacetylation by members of the superfamily of HDACs is necessary for oligodendrocyte precursor cell (OPC) differentiation into mature oligodendrocytes (OLs). However, it remains unknown if transient HDAC inhibition may promote OPC survival as has been shown in other cell types. To investigate HDAC expression and activation patterns specifically in mouse OPCs, we developed a novel primary culture of enriched $O4^+$ OPCs from mouse pup cortices. In co-culture with rat dorsal root ganglia (DRG), OPCs align with DRG neurites and mature into $O1^+$/myelin basic protein $(MBP)^+$ OLs . We observe differences in the HDAC expression pattern of multiple HDAC isoforms in OPCs relative to their terminally differentiated phenotypes, OLs and astrocytes. To determine the role of HDAC activity *in vitro*, we treated OPCs with the FDA approved pan-HDAC inhibitor suberoylanilide hydroxamic acid (SAHA). Surprisingly, OPCs treated with SAHA up to 72 hours resulted in approximately 65% reduction in cell survival relative to vehicle-treated OPCs. Similar treatment with SAHA of astrocytes and OLs derived from enriched $O4^+$ OPCs resulted in 25-30% loss of cell survival; likely a loss of OPCs that have yet to fully differentiate. SAHA-mediated death of OPCs induces apoptosis as evidenced by caspase activation 24 hours following treatment. OPC survival improves when SAHA treatment is accompanied by treatment with the potent general caspase inhibitor Q-VD-OPH. Corroborating our *in vitro* findings, *in vivo* 48 hour SAHA treatment of postnatal day 7 CNP-EGFP transgenic mouse pups significantly increased the number of cleaved caspase 3 $(CC3)^+$ apoptotic cortical OPCs compared to vehicle treated pups ($p < 0.05$). SAHA is currently involved in more than 180 clinical trials, some of which include a pediatric patient population, and is in consideration for use in the treatment of psychiatric and neurodegenerative conditions. These results strongly suggest that such clinical use of SAHA may negatively impact OPC survival and be detrimental to the myelinating brain and spinal cord.

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Systemic Isotretinoin Therapy Normalizes Exaggerated TLR-2-Mediated Innate Immune Responses in Acne Patients

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Acne is a common human ailment that can cause significant physical and psychological morbidity. Though it is thought to be partially caused by *Propionibacterium acnes* colonization, studies suggest that patients' specific immune responses to *P.acnes* may play a larger role in acne than the pathogenicity of *P.acnes* itself. Isotretinoin, a pro-drug for retinoic acid, is the only agent that induces a permanent remission of acne, but the mechanism underlying its long-term efficacy is unknown. We hypothesized that modulation of the immune response to *P.acnes* is key to isotretinoin's ability to induce long-term or permanent remissions of acne. Peripheral blood samples were obtained from acne patients before starting isotretinoin therapy (baseline) and at 1, 4, 8, and 20 weeks after starting isotretinoin therapy. Monocytes and lymphocytes were isolated, activated *in vitro*, and analyzed by flow cytometry. Acne patients' monocytes expressed higher levels of TLR-2 and secreted more IL-1β, IL-6, IL-8, IL-10, and IL-12

in response to *P. acnes* than monocytes from normal volunteers. Isotretinoin therapy significantly decreased TLR-2 expression and inflammatory cytokine secretion by acne patients' monocytes compared to baseline. These changes continued through 20 weeks of isotretinoin therapy and persisted for at least six months after the cessation of therapy, indicating that modification of patients' immune responses to *P. acnes* may represent a long-term mechanism by which isotretinoin can cure acne. Because the exogenous administration of retinoids causes severe side-effects including birth defects, studies characterizing the action of isotretinoin in humans *in vivo* are lacking. This is the first study to show that isotretinoin has immunomodulatory effects in acne patients *in vivo*. Findings from this study extend to other diseases for which isotretinoin is used as a therapy as well as inflammatory disorders characterized by TLR-2 over-expression.

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The Role of Glo1 in Anxiety-Like Behavior

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Anxiety disorders affect over 40 million adults each year. Glyoxalase 1 (*Glo1*) is a gene that has been implicated in anxiety in mouse models. To establish a causal role for Glo1 in anxiety-like behavior, we generated BAC transgenic mice that overexpress *Glo1*. Transgenic mice have increased anxiety-like behavior. We also synthesized an inhibitor of Glo1 to investigate whether Glo1 inhibition reduces anxiety-like behavior in mice. Finally, we have investigated the molecular mechanism underlying Glo1's effect on anxiety. Glo1 is the critical enzyme for detoxifying methylglyoxal (MG), a byproduct of glucose metabolism. As such, we investigated MG as the relevant intermediate regulating anxiety-like behavior. Current research is focused on establishing the molecular mechanism through which MG might affect anxiety.

50 (Cancelled)

Alpha-Catenin Phosphorylation in a C-Terminal Phospho-Domain Affects Conformation and Adhesion-Dependent Processes

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Cell-cell cohesion is a defining feature of multicellular organisms, and some cancer types are driven by defects in the structural 'Velcro' that holds cells to one another. It is widely viewed that the cadherin/catenin adhesion complex is a master regulator of this adhesive "Velcro." This complex comprises a transmembrane "cadherin" component that mediates calcium-dependent homophilic recognition, and a number of associated "catenins" that link cadherins to the underlying cytoskeleton. As the sole actin-binding component of the cadherin/catenin complex, alpha-catenin (α-cat) is viewed as the key linker protein between adhesion receptors and the actin cytoskeleton. Using a mass spectrometry based phosphoproteomic analysis, our lab has discovered a highly conserved phosphorylation domain of five serine/threonine residues in α-cat that is situated just N-terminal to its F-actin-binding site. These phosphorylations also lie within the C-terminal end of the mechano-sensitive, auto-inhibitory region of α-cat that restricts recruitment of vinculin, another actin-binding protein, to the cadherin/catenin complex (Yonemura, 2010, *NCB).* Radioactive 32[P]-labeling studies confirm that these sites are the major targets of phosphorylation in cells. I have performed *in vitro* 32[P]-ATP kinase assays on recombinant, purified GST-tagged α-cat to determine that these sites are targeted hierarchically by casein kinases (CK) 2 and 1. Preliminary studies indicate that phosphorylation at S652, S655, and T658 by CK1 depends on the status of the CK2 site, S641, suggesting that phosphorylation by CK2 "primes" α-cat for phosphorylation by CK1. In addition, the CK1 site is less accessible in the full-length α-cat protein, suggesting that combinatorial phosphorylation by CK1 and CK2 alters the conformation of α-cat. We hypothesize that phosphorylation of α-cat at S641 by CK2 enhances phosphorylation by CK1 at S652, S655, and T658, resulting in a more open conformation that favors α-cat binding to vinculin and F-actin.

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In Vivo Real Time Observations of Immune Cells and Neurons in Traumatic Spinal Cord Injury

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The *in vivo* characteristics of immune mediated secondary axonal dieback after traumatic spinal cord injury are not known. Studies of interactions between DRG neurons and macrophages in culture show a cell-cell contact mediated interaction that causes a dramatic retraction response by the neuron (Horn and Busch et al. 2008). Although these cell types are in contact in tissue, this type of dramatic retraction does not occur. Time lapse multi-photon imaging methods provide a powerful tool for observing these cellular interactions in real time in intact tissue with minimal disruption and high resolution. Animals with genetically expressed fluorescent proteins allow us to observe cellular structures without staining protocols. Yellow fluorescent protein expressed in a neuronally restricted manner under the Thy-1 promoter (Feng et al. 2000) labels single axons in the living animal. Green fluorescent protein expressed in place of the fractalkine receptor CX3CR1 (Jung et al. 2000) allows for the observation of monocytic cells and microglia. We have used multi-photon *in vivo* imaging to characterize axonal dieback from a dorsal column crush injury in these double transgenic mice over a period of several days. We have observed infiltration and movement of CX3CR1 GFP positive cells in the parenchyma of the lesion and in the subarachnoid space. Axons with retraction bulbs die back from the lesion by a membrane blebbing mechanism, also seen in response to physical contact by a CX3CR1 positive cell at time points 5-8 days after injury. Axonal retraction balls still exhibit cytoplasmic loss even at three weeks after injury although their gross position within the

lesion remains static at these timepoints. We also demonstrate using bone marrow chimeric mice that CX3CR1 positive radio-resistant cell bodies are largely static after a lesion, while blood derived CX3CR1 positive cells are motile. Understanding the in vivo cellular interactions involved in this secondary axonal injury may lead to candidates for clinical treatment.

Busch SA and Horn KP et al. J Neurosci 29:9967–9976. Feng G et al. 2000. Neuron 28(1):41-51. Jung S et al. 2000. Mol Cell Biol 20(11):4106-14.

Funding - NINDS NS35713 to JS. and Dana Foundation Brain and Immuno-Imaging Award to AYH

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Identification of Mutations in Families with Congenital Heart Disease

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Background: Congenital Heart Disease (CHD) affects more than 1% of newborns and is widely believed to be due to mutations in genes involved in cardiac development. However, to date only about 5% of the genetic causes of CHD is known. **Objective:** Our objective is to study families with CHD from Lebanon, a highly consanguineous population, using a combination of SNP genotyping and next-generation sequencing. **Methods:** Target-capture sequencing of cardiac-enriched genes was performed on more than 150 families with CHD. The phenotypes include septal defects (atrial and ventricular), valvular disease (pulmonary stenosis, aortic stenosis, tricuspid atresia, bicuspid aortic valve), coarctation of the aorta, tetralogy of fallot, transposition of the great arteries, single ventricle, Ebstein anomaly, and atrioventricular canal defects. Mutation-negative large multiplex families were studied using SNP genotyping and whole-exome sequencing. **Results**: Target-capture sequencing analysis of 50 families identified several homozygous missense mutations in cardiac genes (*JAG1*, *NOTCH1*, *MID1*, *NF1*, *NUP188*, *NFATc4*, *FLNA*). We also identified a novel heterozygous frameshift mutation in *NKX2.5* and novel heterozygous missense mutations in *TBX5*, *ELN*, and *TBX20*. Mutation segregation with the phenotype was confirmed in most of the families. Analysis of target-capture sequence data from the remaining 100 families is underway. Linkage analysis mapped the disease locus in multiplex mutation-negative families and exome sequence analysis is underway. **Conclusion**: Dominant mutations occur in consanguineous populations. The combination of SNP genotyping and next-generation sequencing is an expected method to study gene mutations in families with congenital heart disease, particularly those with recessive mutations.

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Novel Structures and a Novel Target for the Marine Derived Napyradiomycins Suggest a Possible Therapeutic Use in Resistant Cancers

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The marine world is a vast underexplored and underutilized resource in the development of novel therapeutic compounds to combat human disease. In the course of exploring the marine pharmacome we have revisted the napyradiomycin class of marine compounds. This expanding is produced by a unique marine biosynthetic pathway, yet very little pharmacological work has been undertaken to explore their biological effects. We report the isolation and structural elucidation of five new members of the napyradiomycin class. In the course of isolation of these molecules we also isolated nine previously described napyradiomycins. Structure activity relationship studies suggest that minor variations in the structure of these compounds may have significant effects on their activity. Through the use of flow cytometry, we were able to show that napyradiomycin cytotoxicity results from the induction of apoptosis. The results of the structure activity relationship analysis, and the observed induction of apoptosis, suggests a specific protein target for these molecules. Using semi-synthesis a molecular probe was created with two of the napyradiomycin molecules combined with a coumarin flurophore showing localization of the molecule in the endoplasmic reticulum of cultured cells. Subsequently this same probe was used to immunoprecipitate a protein target revealing an HSP90 molecule, likely GRP 94 due to the localization in the endoplasmic reticulum. As GRP 94 is upregulated in many cancers and is associated with increased chemotherapeutic resistance these molecules may represent a novel therapeutic approach to target chemotherapy resistant tumors.

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Activated Hepatic Stellate Cells Upregulate Transcription of Ecto-5'-Nucleotidase/CD73 via Specific SP1 and SMAD Promoter Elements

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Adenosine is a potent modulator of liver fibrosis and inflammation. Adenosine has been shown to regulate such diverse activities as chemotaxis, contraction, and matrix production in hepatic stellate cells (HSC). CD73 is the rate-limiting enzyme in adenosine production. A recent manuscript demonstrated that *Cd73*-deficient mice are resistant to experimental liver fibrosis and have impaired adenosine generation. However, cell-specific expression and regulation of CD73 within the fibrotic liver have not been defined. In particular, prior evidence demonstrating that liver myofibroblasts, the cells believed to be responsible for matrix formation in the liver, express CD73 is lacking. Thus, we tested the hypothesis that HSC and portal fibroblasts (PF), cells that undergo differentiation

into liver myofibroblasts, express CD73 in a regulated fashion. We found that CD73 is weakly expressed in quiescent HSC and PF, but is markedly upregulated at the transcriptional level in myofibroblastic HSC and PF. We furthermore found that CD73 protein and its functional activity are strongly increased in fibrous septa in rats subjected to experimental fibrosis. To determine the mechanism for the upregulation of *Cd73* gene, we cloned the rat *Cd73* promoter, and then used serial truncation and site-directed mutagenesis to identify key regulatory elements. We identified two consensus SP1 motifs and one SMAD binding site, each of which was necessary for *Cd73* gene upregulation. In conclusion, activated HSC upregulate *Cd73* gene expression, via specific SP1 and SMAD promoter elements, after myofibroblastic differentiation. The ecto-5'-nucleotidase/CD73 enzyme is a novel cellular marker of activated liver myofibroblasts *in vivo* and *in vitro*, and thus represents a promising molecular target for anti-fibrotic therapies in liver diseases.

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Markers of Nonselective and Specific Activation of NK Cells

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Natural killer (NK) cells are innate immune lymphocytes that are important for protection against infections and tumors. Once activated, NK cells can proliferate, secrete cytokines and kill cells. Mouse models and cases of human NK cell deficiencies have shown that NK cells are especially important for the protection against herpes virus infections, such as cytomegalovirus (CMV). CMVs are species specific, and murine CMV (MCMV) infection of mice is used as a model for human CMV (HCMV) infection of humans. Furthermore, the extensive study of MCMV infection and NK cell activation has made it an invaluable model for understanding the biology of NK cells. There are distinct phases of NK cell activation during MCMV infection: the nonselective phase is driven by pro-inflammatory cytokines, and the specific phase is driven by recognition of a virally encoded protein, m157, by an NK cell activation receptor, Ly49H. We identified markers of specific activation on NK cells during MCMV infection based on differential expression patterns on $Ly49H^+$ and $Ly49H^-$ NK cells. We determined that stem cell antigen 1 (Sca-1) is a novel marker of both nonselective and specific NK cell activation. We have also further characterized the killer cell lectin-like receptor G1 (KLRG1) and CD27 as markers of specifically activated NK cells. By infecting mice with a virus that does not express the ligand for Ly49H (MCMV-Δm157), we have confirmed that the differential expression of Sca-1, KLRG1, and CD27 on $Ly49H^+$ and $Ly49H^-$ NK cells is dependent on Ly49H recognition of the m157 protein. The identification of markers on specifically activated NK cells will allow us to study differences betweens specifically and non-specifically activated NK cells during MCMV infection. Furthermore, these markers will allow us to study specific NK cell activation in settings where activation receptors remain unknown.

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Staphylococcal MSCRAMMs Bbp and SdrE are Allelic Variants that are Associated with Species Tropism

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Multidrug-Resistant *Staphylococcus aureus* (MRSA) is a commensal bacterium that causes a wide array of diseases. Additionally, the presence of MRSA in livestock and the raw food they produce has become a growing concern. This raises the question of how it is that MRSA can infect such a diverse set of hosts. Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs) on *Staphylococcus aureus* play roles in attachment, invasion and immune evasion. It has been recently been reported by our lab that one of these MSCRAMMs, Bbp, binds to human fibrinogen.

We show that *bbp* can be considered an allelic variant of another MSCRAMM, *sdrE*, due to gene presence, location and similarity. While the two proteins are 87% identical overall, we were surprised to find that most of the variance occurs in the ligand binding domain, which only has 66% sequence identity. Molecular epidemiological evidence shows that while *bbp* is found in 30-40% of human Staphylococcal strains, it is almost entirely absent from animal Staphylococcal strains. We show through basic and advanced *in vitro* biochemical techniques that Bbp shows a much higher affinity for human fibrinogen than SdrE and specifically binds to fibrinogen only from humans. In contrast, SdrE shows varying affinity for fibrinogen from a wide array of animals. This finding provides cause to the epidemiological presence of Bbp and SdrE in human and animal Staphylococcal strains.

The structural basis for this difference is elucidated by solving the crystal structures of apo-Bbp and apo-SdrE as well as co-crystals of Bbp with a peptide representing the ligand binding sequence from the human fibrinogen Aα chain. Mutations in the recombinant proteins were made based on the solved structures for confirmation of structure and to discover if certain residues could be responsible for the difference in binding profile. These point mutations and a Bbp/SdrE Chimera show that the Lock Region of the two proteins is a major cause of this difference in binding profile and species tropism.

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Measles Immunity and Measles Vaccine Acceptance among Healthcare Workers in Paris, France

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Background: A measles outbreak is ongoing since 2008 in Europe, including France. Unprotected healthcare workers (HCWs) may contract and spread the infection to patients. The objective of this study was to evaluate HCWs' measles immunity and vaccine acceptance in our setting. **Methods/Results:** In a survey-based study conducted in 3 university hospitals in Paris (France), 353 HCWs were included between April 27, and June 30, 2011. The following

data were collected at inclusion: age, hospital unit, occupation, history of measles infection, measles vaccination (number of doses and dates), previous measles serology and acceptance of a measles vaccination in case of seronegativity. Sera were tested for the presence of specific anti-measles IgG and IgM antibodies using the "Captia® measles" ELISA assay (Trinity biotech, USA). The mean age of the participating HCWs was 36 years old [range 18-67] and 280 (79.3%) were women. One hundred and five persons (29.8%) declared a history of measles, and 90 (25.5%) declared never having received a measles vaccination. Among the 353 HCWs included in the study, 324 (91.8%) were immunized against measles (IgG above 90mIU/ml). The risk factors for being unprotected were age (18-24 years, OR 11.8, 95% CI [2.4-58.4] and 25-34 years, OR 8.4, 95% CI [1.8-38.4] compared > 35 years), absence of history of measles and absence of vaccination. The global acceptance rate for a measles vaccination, before knowing their results was 80.8%. **Conclusions:** In this cohort of HCWs, 8.2% were susceptible to measles, mostly among the youngest (<35 years). Vaccine acceptance against measles was high. Vaccination campaign in healthcare settings should target specifically healthcare students and junior HCWs.

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Top 10 Common Traits among Hiroshima/Nagasaki Atomic Bomb Survivors; What can we Learn from History?

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Atomic bombs dropped in Hiroshima and Nagasaki in 1945 were one of the most horrible disasters in human history. They instantly killed at least 120,000 people and injured more than 100,000 others. After several decades of restoring and recovering, many survivors are still struggling to forget and forgo the physical, emotional and/or psychological scars in their lives, although they were fortunate enough to survive the bombs and enjoy longevity. Then, why are they able to survive and strive while others have died instantly or from gradual radiation effects? The purpose of this study is to explore the dietary and life-style factors that are common among the atomic bomb survivors. The data comes from the interviews of 30 atomic survivors, who are recognized as atomic bomb victims, *hibakusha,* by the local government. Dietary and health style factors that are common in most of the interviewees are summarized into ten categories. 1) Indirect radiation exposure: 77% of survivors had escaped from direct radiation exposure, and those who did have direct exposure had burns that were not extensive. 2) Psychological factors: All interviewees had positive attitudes toward life without complaints about their dire destiny. Among others, many Nagasaki survivors had strong religious faith. 3) Miso: 93% of the interviewees ate miso or miso soup every day, and scientific research has proven the irradiating effects of miso.4) Food intake amount: Since it was wartime, the food was scarce. All of the survivors had to endure with scarce food intake. 5) Country-side environment: 44% of the interviewees moved to the countryside after bombing, so they had enough food to eat, and the environment was not as contaminated as in the city. 6) Unprocessed food: 97% of the interviewees ate whole grain and no processed food. 7) Vegan: All interviewees ate a lot of vegetables and beans, and 93% of interviewees did not eat any meat around the time of radiation exposure and for a long time afterwards. Their diet was almost vegan. 8) Sugar intake: No interviewees ate any sugar and few ate sweets of any kind.9) Bed ridden: The survivors who suffered from burns were bedridden and tended to live longer than those who survived and had to work immediately to help others. 10) Smoking: No interviewees smoked entire life. **Conclusions:** Although some of the traits mentioned above could have been observed among those who did not survive, many of these traits may have increased survival rate. This study has delineated common dietary and life-style traits of Atomic bomb survivors. After the Tsunami hit the northeast area of Japan in March 2011, radiation contaminated the area. Further study will be needed to see if these 10 traits are also consistent with the Tsunami survivors with radiation exposure.

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Ang Converting Enzyme Inhibition *vs.* Angii Receptor Blockade in Marfan Mouse: Role of Bradykinin

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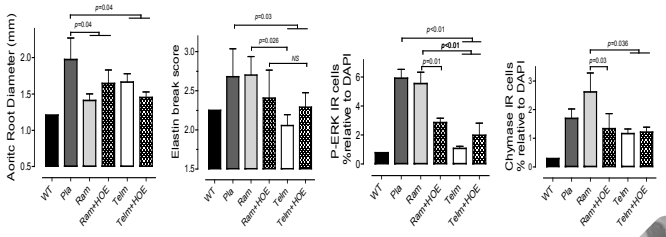
Angiotensin receptor blockers (ARB) reduces aortic aneurysm in the *Fbn1*$^{C1039G/+}$ mouse model of Marfan syndrome, by interfering with angiotensin II (Ang II) activation of transforming growth factor beta (TGF-β) signaling pathways. Angiotensin converting enzyme inhibitors (ACEi) decrease the formation of Ang II; but also increase bradykinin which can activate the TGF-β pathway. To test the hypothesis that ACEi and ARBs differ in their effect on aortic aneurysm development in *Fbn1*$^{C1039G/+}$ mice, we compared 12-week treatment with telmisartan (3 mg/L and 24 mg/L), or ramipril (30mg/L) in the presence or absence of the bradykinin B_2 receptor antagonist HOE-140 (0.1 mg/Kg/day). ARB and ACEi reduced aortic root diameter but only ARB prevented elastin disruption, decreased mast cell infiltration and P-ERK immunoreactivity in the ascending aorta. HOE-140 abolished the difference in the effect of ARB and ACEi on elastin degradation, and decreased mast cell infiltration and P-ERK immunoreactivity in the aorta of mice treated with ACEi. In the lung, ARB treatment prevented the development of emphysematous changes, decreased mast cell infiltration and decreased MMP12 and MMP9 activity compared to ACEi treatment. HOE-140 reduced MMP12 activity in the lung of mice treated with ACEi or ARB but did not reduce mast cell infiltration or emphysema. Endogenous bradykinin contributes to different effects of ACEi and ARB on elastin disruption in the aortic root by promoting activation of P-ERK and mast cell infiltration (Figure). Clinical studies are required to evaluate the comparative efficacy of ACEi and ARB in Marfan syndrome.



Poster Abstracts

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Tbx1 is a Key Mediator of Tooth Size, Shape and Enamel Formation during Tooth Development via Interactions with Pitx2 and Dlx2

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Objective: The T-box transcription factor TBX1 is currently the candidate gene for DiGeorge Syndrome (DGS) due to its location in the 22q11.2 critical region. *TBX1* is deleted in virtually all DGS patients, and *Tbx1*$^{-/-}$ mice contain phenotypes similar to patients of DGS, including hypoplasia of thymus and parathyroid, cardiac outflow tract abnormalities, abnormal facies and cleft palate. Studies have shown DGS patients often have tooth defects such has enamel hypoplasia and hypomineralization, hypodontia, delayed tooth eruption and excessive dental caries. Previous research using mouse model has linked a decrease in *Tbx1* with a decreased production in amelogenin. However, the long-term effects of *Tbx1* on enamel formation are unclear due to the embryonic lethality of *Tbx1*$^{-/-}$ mice. Previous research has also shown that Tbx1 physically interacts with other transcription factors such as the C-terminus of Pitx2. The aim of this study is to fully understand the how Tbx1 physically interacts with other transcription factors and its role in enamel formation when *Tbx1* is conditionally knocked out in the epithelium. **Methods:** Wild type (WT), K14Cre*Tbx1*$^{flox/flox}$, Pitx2Cre*Tbx1*$^{flox/flox}$ mice at embryonic day E14.5, E16.5, E18.5, P0, and P4 were harvested, processed, and embedded in paraffin. H&E staining and immunohistochemistry (IHC) for amelogenin, ameloblastin, and Ki67 were done on 7 micron sections. MicroCT and SEM were done on skeletal preps prepared at P20. Transfections in Chinese hamster ovarian (CHO) and oral epithelium (LS8) cell lines, GST-pull down and Western blots were performed to understand how Tbx1 interacts with transcription factors Pitx2 and Dlx2. **Results:** Conditional knockout of *Tbx1* in both K14Cre and Pitx2Cre leads to a decrease in cell proliferation of ameloblast cells, resulting in smaller teeth and decreased enamel formation at E14.5, E16.5, E18.5, P0 and P4. In the mutant mice, preameloblasts cells were not as polarized or elongated compared to the WT mice. Moreover, the cervical loop was smaller and contained fewer cells in the mutant mice. From the SEM, it was evident that the enamel layer was thinner and the enamel matrix prisms were more loosely packed in the mutant mice. In addition, a decreased cusping of the first two molar teeth were observed but the third molar developed faster in the mutant mice. *In vitro*, Pitx2 binds to the N terminus of Tbx1, and without the N terminus, Tbx1 is unable to attenuate activation of the Pitx2 promoter by Pitx2. Transfections also show that *Tbx1* attenuates the activation of the *Amelogenin* promoter and *Dlx2* promoter by *Dlx2*. **Conclusions:** *Tbx1* regulates tooth size and shape and is an important regulator of amelogenesis by altering the activity level of other transcription factors such as Pitx2 and Dlx2.

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Correlating Cardiopulmonary Dysfunction in a Mouse Model of Muscular Dystrophy

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Background: The muscular dystrophies are marked by progressive muscle degeneration and subsequent maladaptive repair. In the most common childhood form, Duchenne muscular dystrophy (DMD), patients are wheelchair bound by their teens and die of cardiac and/or pulmonary complications in their third or fourth decade without mechanical support. Mutations in dystrophin lead to DMD, and mutations in the dystrophin associated proteins, the sarcoglycans, lead to limb girdle muscular dystrophy that is similar to DMD. The *Sgcg* mouse that lacks γ-sarcoglycan is an excellent model of muscular dystrophy replicating many characteristics of the human disease. **Methods:** We characterized *Sgcg* mice on a mixed genetic background to assess correlation between cardiopulmonary dysfunction and skeletal muscle pathology. More than 250 Sgcg mice derived from an F4 intercross of Sgcg^{D2} and Sgcg^{129} mice were studied at 8 weeks of age. Mice underwent echocardiography to measure left ventricular function and pulmonary artery acceleration time as a measure of right ventricular function. Mice were injected with the vital tracer Evans Blue dye to mark muscle degeneration, and then sacrificed. Multiple muscle groups were studied for dye uptake using a quantitative spectrophotometric assay. Other muscle groups, including the right and left ventricles were subjected to hydroxyproline measurements to quantify fibrosis. Pearson correlations were determined between echocardiographic and pathological parameters. **Findings:** Reduced fractional shortening or ejection fraction correlated with increased left ventricular diameter. A positive correlation was found between heart rate and cardiac output. We found that dye uptake into the abdominal muscles, as an indicator of muscle damage, correlated with mean and peak velocities measured at the pulmonary valve (PV). PV velocities correlated negatively with pulmonary artery acceleration time, an indirect measure of pulmonary hypertension. Fibrosis in the abdominal muscles negatively correlated with fibrosis in the diaphragm and right ventricles. **Conclusions:** Cardiopulmonary dysfunction in mouse models parallels what occurs in human patients. Left ventricular functional decline is accompanied by dilation and an increase in heart rate to maintain cardiac output. Diaphragm and abdominal muscles both contribute to respiratory function. Respiratory dysfunction contributes to impaired right ventricular function. These quantitative data delineate the sequence of pathology in muscular dystrophy and set the stage for mapping genetic loci that regulate these parameters.

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Oncolytic HSV-1 Expressing Interleukin-15 for Brain Tumor Therapy

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Conditionally replication-competent oncolytic herpes simplex type-1 virus (oHSV) vectors are promising therapeutics for malignant gliomas. Our laboratory group recently completed

a microarray analysis of glioma samples from a phase Ib clinical trial in which patients with malignant gliomas were administered oHSV. In samples obtained from patients with a positive response, transcript levels of the immunostimulatory cytokine interleukin-15 (IL-15), all receptors necessary for optimal IL-15 signaling, and transcripts indicating the presence of cytotoxic lymphocytes responsive to IL-15 ($CD8^+$ T and natural killer [NK] cells) were significantly increased. As immune responses can work in concert with oHSV lytic replication to combat tumors, we sought to determine whether IL-15 enhances oHSV efficacy. We hypothesize that IL-15 propagates $CD8^+$ T and NK cell anti-glioma immune responses, thus jointly contributing to tumor reduction with oHSV lytic replication. To investigate this hypothesis we constructed oHSV vectors expressing murine (m)IL-15 alone or with the IL-15 receptor α (IL-15Rα), which is necessary for IL-15 presentation and signaling. These vectors replicate identically, indicating that the insertions do not alter the direct lytic activity of the vectors. The vector encoding mIL-15 alone produces a low level of mIL-15, whereas the vector dually encoding mIL-15/IL-15Rα substantially improves mIL-15 production. The dual expressing vector also produces and secretes the physiologically relevant complex of mIL-15 bound to mIL-15Rα (3ng/mL). Both vectors remain aneurovirulent (LD_{50} > $1x10^7$ plaque forming units, comparable to other oHSV vectors). Dual mIL-15/IL-15Rα expression from oHSV improved survival over saline in preliminary survival studies using an aggressive murine brain tumor model (median survivals: saline – 14 days; mIL-15/IL-15Rα oHSV – 25 days. p = 0.001), whereas mIL-15 expression alone did not (median survivals: saline – 14 days; mIL-15 oHSV – 17 days. p = 0.1128). In vivo studies investigating the stimulation of bystander immune-mediated tumor clearance by oHSV-produced mIL-15/IL-15Rα in murine models of malignant glioma are underway. Results of these studies may support investigation of oHSV expressing human IL-15/IL-15Rα in malignant glioma clinical trials.

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CCR1 Antagonists Inhibit Osteoclastogenesis

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Background: Osteolytic bone disease (OBD) is a hallmark of Multiple Myeloma (MM), a cancer of plasma B-cells. OBD results from uncoupling of osteoclast (OC) and osteoblast activity within the bone marrow microenvironment. A vicious cycle of cytokine signaling between MM and OC cells underlies this perturbation of bone metabolism, with the chemokine receptor CCR1 playing a key role. Since both MCs and OCs express CCR1, we examined whether CCR1 antagonists hold promise in the treatment of MM and OBD. **Hypothesis**: We hypothesized that inhibition of CCR1 through small-molecule antagonists would reduce both OC differentiation and OC activity. **Methods**: OCs were differentiated from human peripheral blood mononuclear cells (PBMC) in the presence or absence of two CCR1 antagonists, MLN-3897 and PS31291. Proliferation of differentiating PBMCs was measured using AlamarBlue. Production of IL-6, a modulator of B cell activity, was measured using ELISA. The presence of mature, multinucleated OCs was assessed microscopically using hematoxylin and tartrate-resistant acid phosphatase (TRAP) stains. Finally, the ability of antagonists to diminish MM cell migration in OC-derived media was evaluated. **Results**: Data from AlamarBlue assays, confirmed by microscopic visualization of TRAP staining, demonstrated that CCR1 antagonists inhibit differentiation OCs in a dose-dependent manner. Data from ELISA experiments show a concentration-dependant decrease in IL-6 production. Finally, migration assays demonstrated that antagonists block MM chemotaxis towards OC-derived conditioned cell media. **Conclusion**: Preliminary review of our data indicates that CCR1 antagonists may provide a mechanism by which OC differentiation, viability, and activity can be inhibited. Furthermore, we have established strong evidence that these antagonists also have the capacity to blunt signaling and functional interaction between OC and MM cells. Therefore, CCR1 antagonists may potentially serve as a single therapy that can mediate dual effects in myeloma patients–inhibiting cancer progression and mitigating OBD.

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Mechanisms of Protein Localization and Retention within the Cone Photoreceptor Sensory Cilium

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Purpose: In rod outer segments (OS), localization and retention of transduction proteins is thought to be mediated by targeted, active transport and association with OS disc membranes that are physically separate from the plasma membrane. Targeting and retention of signaling proteins within cone OSs is not understood. Unlike rods, cone OS discs are contiguous with the plasma membrane. Therefore retention of membrane-associated proteins appears to require diffusion barriers either at the cilium base, as has been suggested for primary cilia, or at the disc rims. We examined a putative cone opsin OS targeting signal that is analogous to that on rod opsin, and the possibility of diffusion barriers at the ciliary transition zone and disc rims, as possible mechanisms for protein localization and retention in the cone OS. **Methods:** Transgenic *X. laevis* that express EGFP fused with lipidation motifs, red cone opsin (XRCOP), or a putative XRCOP OS localization sequence, in cones under the *Xenopus* red cone opsin (XRCOP) promoter, were created using the REMI method. Confocal and multiphoton imaging of live cones in retinal slices were used to study protein localization and dynamics. **Results:** Lipidated-EGFP was found in both of the major photoreceptor compartments, while XRCOP-GFP was localized to the OS. EGFP fused to the C-terminus of XRCOP failed to localize to the OS. Although both integral and peripheral membrane proteins diffused laterally and axially in the OS, diffusion of opsin-GFP between discs was significantly retarded relative to lipidated-EGFP. **Conclusions:** Our results are consistent with cone opsin retention in the OS being mediated by a diffusion barrier at the ciliary transition zone. There appears to be a sieving mechanism at the disc rims that impedes the movement of integral membranes proteins between discs to a greater extent than peripheral membrane proteins. The fact that lipidated GFPs are found in both IS and OS compartments suggests either that the barrier at the ciliary transition zone is selective for intrinsic membrane proteins, or that lipidated GFPs dissociate from membranes and equilibrate between these compartments. Unlike rod opsin, addition of the C-terminal 40 amino acids of XRCOP

was not sufficient to localize EGFP to the OS, possibly due to the absence of palmitoylation sites in the cone opsin sequence.

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Interleukin-1 and Platelets as Key Drivers of Cerebrovascular Inflammation

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Background: The infiltration of neutrophils across vascular endothelium into tissue contributes to neurological disease, including in cerebral ischaemia. *In vitro* evidence suggests a key role for platelet-derived interleukin-1alpha (IL-1α) in mediating neutrophil migration across the cerebrovascular endothelium. This study investigates the role of platelets and IL-1 using *in vivo* models of vascular inflammation.
Method: Three murine models of neutrophil migration were used. Bacterial endotoxin, lipopolysaccharide (LPS), was injected intraperitoneally or into a dorsal air pouch. Lavage of the respective cavities was performed 6 hours later. LPS was stereotactically injected into the striatum, and the brain fixed and removed 24 hours later. Flow cytometry or immunohistochemistry were used to assess migrated neutrophil numbers. To determine the role of platelets, platelet depletion was induced via an anti-CD41 antibody. To determine the role of IL-1, IL-1α/β knockout mice were used. Cytokines were quantified using cytometric bead array. **Results:** In all models, platelet depletion abolished the neutrophil migration, indicating a key role for platelets in this process. A robust inflammatory response was seen in serum cytokines after LPS injection and platelet depletion selectively abrogated the increase in serum IL-1α. IL-1α/β knockout mice revealed a significant difference in neutrophil migration between knockout animals and controls in the encephalitis model, with no difference in the peritonitis model. **Conclusion:** In an *in vivo* peritonitis model, neutrophil migration appears to be dependent on platelets, yet independent of IL-1. In an encephalitis model, neutrophil migration appears dependent on platelets and IL-1, raising the possibility of tissue-specific inflammatory pathways.

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Exploring the Role of Activity Dependent Mrna Processing Of Orb2A in Long-Term Memory Formation

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Lasting, experience dependent changes in synaptic connections underlie the cognitive adaptations that are central to the human mind; dysregulation of changes in synaptic connectivity likewise contribute to many neurological diseases, ranging from Post-Traumatic Stress Disorder to Autism Spectrum Disorders. While the molecular mechanisms that stabilize synaptic changes in response to activity remain unknown, studies have implicated a translational regulator, cytoplasmic polyadenylation element binding protein (CPEB), in long-lasting activity dependent alterations of synaptic connections on the sub-cellular and behavioral levels. The *Drosophila melanogaster* CPEB gene, o*rb2*, which encodes 2 major neuronal isoforms, Orb2A and B, is haploinsufficient for long-term memory formation. Moreover, Orb2 forms amyloid-like oligomers in response to neuronal activity in the adult fly brain. Although the oligomers are composed of Orb2A and Orb2B, removal of Orb2A leads to a reduction in oligomerization. Additionally, a substitution mutation in Orb2A (F5Y) that slowed oligomer formation in response to neuronal activity also led to defects in the ability of flies to form persistent long term memory. Together these data suggest a model whereby Orb2A induces oligomerization of Orb2B and this oligomerization is critical for the persistence of memory. Additionally we have observed that the level of Orb2A is very low in the fly brain and that unlike most mRNAs, including Orb2B mRNA, the majority of Orb2A mRNA in the adult fly brain retains a stop-codon containing intron. Based on these observations we hypothesize that retention of the Orb2A intron allows for post-transcriptional regulation of Orb2A expression and that in response to behaviorally relevant stimuli Orb2A mRNA is spliced to produce a translationally competent mRNA which is necessary for persistent long-term memory. This study finds that Orb2A mRNA with the retained intron is the predominant form of the Orb2A transcript under normal lab culture conditions and that splicing of the intron is induced by external cues. Our data suggest a model whereby post-transcriptional mRNA processing is a regulatory step in long-term memory formation.

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Variants in the 3'Untranslated Region of the *KCNQ1*-Encoded Kv7.1 Potassium Channel Strongly Modify Disease Severity in Patients with Type 1 Long QT Syndrome in an Allele-Specific Manner

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Introduction: Heterozygous mutations in *KCNQ1* cause type 1 long QT syndrome (LQT1), a disease characterized by heart rate-corrected QT interval (QTc) prolongation and an increased risk of life-threatening arrhythmias. It is unknown why disease penetrance and expressivity is so variable between individuals hosting identical LQT1-causative mutations. Here, we hypothesized that this variability can be explained by single nucleotide polymorphisms (SNPs) in the 3'untranslated region (3'UTR) of the *KCNQ1*-encoded Kv7.1 tetrameric channel.
Methods and Results: 168 LQT1 patients (96 females, mean age 36 ± 22 yrs, mean QTc 449 ± 40 msec, range QTc 354 to 592 msec) were genotyped for SNPs in *KCNQ1*'s 3'UTR, and six SNPs were found. *In silico* analyses using the miRanda algorithm predicted that the minor variants of SNPs rs2519184 (A) and rs8234 (G) create two miRNA binding sites for miR-378. Real-time polymerase chain reaction (PCR) indicated that miR-378 is expressed in the heart. Dual-reporter luciferase assays and microRNA overexpression studies showed that miR-378 suppresses the expression of the minor AG 3'UTR haplotype but not the major GA 3'UTR haplotype.

Allele-specific quantitative PCR and Western blots showed that individuals heterozygous for the minor AG 3'UTR haplotype had significantly lower *KCNQ1* mRNA and Kv7.1 protein levels in the heart. Accordingly, compared to LQT1 cases homozygous for the major GA 3'UTR haplotype (n = 83, mean QTc 444 ± 32 msec, symptomatic 18%), cases with the minor AG 3'UTR haplotype on their mutant *KCNQ1* allele had 15 ± 27 msec shorter QTc (n = 26, P < 0.01) and experienced less symptoms (15%, P = NS), while cases with the minor AG haplotype on their normal *KCNQ1* allele had 54 ± 45 msec longer QTc (n = 9, P < 0.005) and experienced more symptoms (44%, P < 0.05). **Conclusions:** Our data indicate that SNPs in the 3'UTR of *KCNQ1* function as potent modifiers of LQT1 disease severity. The allele-specific effects on disease severity and gene expression strongly suggest that these SNPs are functional variants that directly alter the expression of the allele on which they reside, and thereby influence the balance between channel subunits stemming from either the normal or the mutant *KCNQ1* allele.

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Protein Altering Variation in Cardiomyopathy Genes in the 1000 Genomes Project

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Background: Hypertrophic and dilated cardiomyopathy arises from mutations in genes encoding sarcomere proteins including *MYH7, MYBPC3, and TTN*. The genetic diagnosis of cardiomyopathy relies on complete sequencing of the gene coding regions, and most pathogenic variation that has been identified is rare. The 1000 Genomes project is an ongoing consortium designed to deliver whole genome sequence information from an ethnically diverse population and is therefore a rich source of both common and rare genetic variants. Phenotypic data is unavailable from subjects in the 1000 Genomes project, and thus genetic variation in this cohort should be considered to include both normal and abnormal individuals. **Methods/Results:** We queried the 1000 Genomes database of 1,092 individuals for exonic variants within the three sarcomeric genes *MHY7*, *MYBPC3,* and *TTN*. Variants were analyzed using several distinct protein prediction algorithms. Sarcomere gene variants were compared to four additional nonsarcomeric genes that were also queried. We focused our analysis on rare protein-altering variation (PAV), or those DNA sequences that predict a distinct protein from the referent genome. We found substantial variation, including protein-disrupting sequences, in *TTN*. We also identified known and predicted pathogenic variation in *MYBPC3* and *MYH7* at a higher frequency than what would be expected based on population-based studies of cardiomyopathy prevalence, including 22 variants described in the Human Gene Mutation Database (HGMD), a comprehensive online database of inherited disease mutations. One variant in *MYBPC3,* S236G, was found at a comparatively higher frequency (MAF 0.09, n=186) supporting that this variant is insufficient to cause disease but may modify disease outcomes in concert with other genetic and nongenetic causes of cardiomyopathy. Of the three sarcomeric proteins, *MYH7* had the greatest variation per kilobase but the least PAV. This indicates that *MYH7* is especially intolerant of PAV and, consequently, these variants are observed infrequently in the population at large. **Conclusions:** The human genome is a rich source of genetic variation, including pathogenic variants. In light of the prevalence of cardiomyopathy in the population at large, single pathogenic variants may be necessary but not sufficient for disease and may need to occur in the context of a genetically susceptible host.

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Smoke Induced Emphysema in Rabbits: Correlation with the Human Disease

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Introduction: Matrix metalloproteinases (MMPs) are highly expressed in the lung during both development and injury. The collagenase MMP-1 and gelatinase MMP-9 are both key proteases implicated in emphysema pathogenesis. Mice lack the gene for MMP-1 and express reduced levels of MMP-9 after smoke exposure, limiting the utility of this model in fully exploring the human disease process. We hypothesize that due to established similarities between rabbits and humans in protease profiles, rabbits exposed to cigarette smoke will potentially develop a disease pattern that parallels what is seen in humans. **Methods:** New Zealand white rabbits (Convance, NJ) were exposed to cigarette smoke 4 hours daily, 5 days per week for 10 weeks (n=5) or 16 weeks (n=5) in a specially designed smoking chamber (Teague Enterprises, CA). Age-matched rabbits exposed to room air for 10 weeks (n=5) or 16 weeks (n=5) served as controls. Following smoke exposure, rabbits were anesthetized, bronchoalveolar lavage (BAL) performed, one lung collected for RNA and protein analysis, and the opposite lung pressure perfused in formalin for histological analysis. Statistical analysis was performed using an unpaired Student's t-test, with $p < 0.05$ considered statistically significant. **Results:** In rabbits, 16 weeks of smoke exposure caused parenchymal lung destruction consistent with emphysema, with a significant increase in mean linear intercept (54.25 μm smoking vs. 34.5 μm control, p = 0.028) and destructive index (83% smoking vs. 50% control, p = 0.001). There was an accompanying increase in macrophages and neutrophils recovered by BAL. Increased cellular apoptosis was detected by TUNEL staining in the lung parenchyma of 16 week smoked rabbits (0.368% smoking vs. 0.1154% control, p = 0.008). Both MMP-1 and MMP-9 were induced in the lung parenchyma after both 10-weeks and 16-weeks of cigarette smoke exposure, detected by Western blot and zymography, respectively. After 16 weeks of smoking, rabbits also exhibited increased TLR4 gene expression measured by real-time PCR. **Conclusions:** The rabbit model of smoking induced emphysema exhibits a lung inflammatory and protease micro-environment closer to that observed in the human disease. This model, which includes the induction of MMP-1 and MMP-9 similar to that seen in humans, will allow us to better understand the mechanism of human emphysema and provide a robust pre-clinical model in which to test therapeutic strategies.

Poster Abstracts

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SMAD Signaling Drives Heart and Muscle Dysfunction in a Mouse Model of Muscular Dystrophy

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Mutations in dystrophin or its associated proteins, the sarcoglycans lead to cardiomyopathy and muscular dystrophy in humans, mice, and flies. The dystrophin complex acts as a molecular rivet, and disruption of the dystrophin complex in muscle causes cellular injury, dysfunction, cell death, and fibrosis. We previously showed that reduction of canonical TGFβ signaling improved walking and heart tube function in a *Drosophila melanogaster* model of muscular dystrophy (Goldstein et al. 2011). We now investigated whether SMAD signaling is elevated in muscular dystrophy in mice, and whether reducing signaling alters disease progression. To study the role of SMAD signaling in injury, we injured the muscle of mice that carry a SMAD-responsive luciferase reporter. Dystrophic muscle from γ-sarcoglycan null mice (*Sgcg*$^{-/-}$) was stimulated ex vivo to contract using a lengthening protocol to produce maximal muscle injury. This protocol resulted in phosphorylation of SMAD2/3, an indicator of SMAD activation. To test whether reducing SMAD signaling improves muscular dystrophy in mice, we introduced a heterozygous null mutation of SMAD4 (Chu et al., 2004) into *Sgcg*$^{-/-}$ mice. Compared to *Sgcg*$^{-/-}$ mice, *Sgcg*$^{-/-}$ *SMAD4*$^{+/-}$ mice showed increased body weight. These mice also showed improved cardiac function, assayed by echocardiography, with increased fractional shortening. These physiologic measures correlate with reduced phenotype severity. We assayed muscle membrane leak and fibrosis using two established assays on multiple muscle groups. *Sgcg*$^{-/-}$*SMAD4*$^{+/-}$ muscle showed extensive Evans blue dye uptake and hydroxyproline deposition, comparable to that seen in Sgcg$^{-/-}$ muscle. We measured muscle fiber size, and found that it was elevated in *Sgcg*$^{-/-}$*SMAD4*$^{+/-}$ mice. These data suggest that functional improvement of *Sgcg*$^{-/-}$ *SMAD4*$^{+/-}$ muscle arises downstream from the primary membrane defect and identify SMAD signaling as a therapeutic target.

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The PI3K Catalytic Subunit p110δ is a Crucial Mediator of Leukemia-Associated Mutant PTPN11-Induced GM-CSF Hypersensitivity

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Juvenile Myelomonocytic Leukemia (JMML) is a rare, lethal disease of young children characterized by expansion of monocyte/macrophage-lineage cells and hypersensitivity to GM-CSF stimulation. JMML is frequently associated with gain-of-function mutations in *PTPN11*, which encodes the protein tyrosine phosphatase, Shp2. Activating Shp2 mutations result in hyperactivation of Class IA PI3K signaling, which, using pharmacologic and genetic approaches, we have found contributes to mutant Shp2-induced GM-CSF hypersensitivity. To investigate the role of p110δ, a PI3K catalytic subunit expressed predominantly in hematopoietic cells, in mutant Shp2-induced GM-CSF hypersensitivity, we used the p110δ-specific inhibitor, IC87114, and a clinical grade pan-PI3K inhibitor, GDC-0941, and found that both reduced GM-CSF-stimulated hyperproliferation and Akt and Erk hyperphosphorylation in macrophage progenitors expressing leukemia-associated Shp2 mutations—both retrovirally transduced Shp2 E76K and endogenously expressed Shp2 D61Y from inducible knockin mice. We crossed mice expressing kinase-dead p110δ (p110δ D910A) with Shp2 D61Y inducible knockin mice, and found that genetic inactivation of p110δ resulted in a significant reduction of mutant Shp2 D61Y-induced GM-CSF stimulated hyper-proliferation and Akt and Erk hyperphosphorylation. To determine if mutant Shp2 induces hyperactivation of PI3K through its scaffolding function (mediated by its N-SH2 and C-SH2 domains), we transduced mouse bone marrow cells with Shp2 E76K mutants bearing genetic disruption of its N-SH2 domain (Shp2 E76K/R32K) or its C-SH2 domain (Shp2 E76K/R138K), and found that both mutations reduced GM-CSF-stimulated Akt hyperphosphorylation and hyperproliferation. Similar studies to investigate the role of mutant Shp2's phosphatase function in regulating PI3K are currently being performed using a Shp2 E76K mutant bearing genetic disruption of the phosphatase domain (Shp2 E76K/ C463A). Together, these studies show that PI3K inhibitors, particularly the newer classes of inhibitors specific for the p110δ catalytic subunit, represent a potential therapeutic strategy for the treatment of JMML.

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The Vascularized Bone Marrow Component is Critical for Donor-Derived Hematopoietic Stem Cell Engraftment, Chimerism and Tolerance in a Murine Hind Limb Transplantation Model

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Background: Vascular Composite Allografts (VCA), such as hand/arm transplants, contain vascularized long bones that instantly and continuously produce bone marrow (BM) cells after engraftment and thus can be considered to be both a VCA and vascularized BM graft. The creation of hematopoietic chimerism through BM transplantation remains the most reliable method for inducing transplantation tolerance. However, the contribution of a vascularized bone graft in VCA towards such effects is largely unknown. Therefore, this study investigates the long-term effects of vascularized BM within VCA under costimulation blockade-based immunosuppression. **Materials and Methods:** Balb/c mice were utilized as osteomyocutaneous (OMC) or myocutaneous (MC, without bone components) VCA donors and C57BL/6 mice as recipients. Immunosuppression consisted of

combined costimulation blockade (anti-CD154, 1 mg IP at day 0 and CTLA4Ig, 0.5 mg IP at day 2) and rapamycin (3 mg/kg/day IP x 7 days, then QOD for 3 weeks) treatment. Viability of BM and efficacy of donor-specific hematopoietic cells engraftment was determined in recipient blood and tissue samples at POD 30, 60, and 120. **Results:** Combined costimulation blockade and Rapamycin treatment significantly prolonged OMC VCA survival (>120 days) as compared to untreated controls (MST 10 days) and CTLA4Ig only treated recipients (MST 36 days). Moreover, OMC VCA showed significantly prolonged survival as compared to MC VCA in either combined costimulation blockade and Rapamycin treated group (MST > 120 vs 87 days) or CTLA4Ig treated group (MST 36 vs 22 days). Macrochimerism could be detected at POD 30 in 4/6 animals, which showed long-term survival, but it tended to diminish and disappeared at POD 60. **Conclusion:** Vascularized BM transplantation is effective in inducing chimerism and VCA tolerance in mice that had undergone short-term combined costimulation blockade and Rapamycin treatment. These findings suggest that vascularized BM plays a critical role in the immunoprevileged features of VCA.

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Emmprin (Cd147) is a Novel Regulator of Invadopodia Formation and Activity

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Background: A defining feature of malignancy is cell penetration through the basement membrane and matrices that separate cellular compartments. Accumulating evidence suggests that invasive cancer cells use specialized structures termed invadopodia, which are actin-based, lipid raft-enriched protrusions abundant in proteases, including membrane-type-1 matrix metalloproteinase (MT1-MMP), cell adhesion molecules, and several signaling proteins. Thus, invadopodia are dynamic cellular depots where diverse cell signaling, vesicular trafficking, and protease activity converge in specialized membrane compartments at the leading edge. Emmprin (CD147), an Ig superfamily protein associated with tumor invasion and metastasis, induces the synthesis of various MMPs. CD147 interacts with various molecules in supra-molecular cell surface complexes, which facilitate cancer progression and therapy-resistance. **Methods**: We employed MCF-10A (non-invasive/non-transformed) breast epithelial cells and invasive MDA-MB-231 breast cancer cells to evaluate the role of CD147 in invadopodia-mediated invasion. CD147 expression was modulated with adenoviruses and siRNA. Invasion was measured with matrigel chambers and gelatin degradation assays. Lipid rafts were evaluated biochemically with density centrifugation and microscopically with conjugated-cholera toxin. Protein interactions were examined using a proximity ligation assay. **Results**: In this study we demonstrate that up-regulation of CD147 is sufficient to induce MT1-MMP expression, invasiveness, and formation of invadopodia-like structures in non-transformed, non-invasive, breast epithelial cells. CD147 up-regulated cells have an increased % of cells with degraded matrix underneath, greater total degradation area, and more active invadopodia. We provide evidence that subpopulations of CD147 and MT1-MMP are in close proximity within these invadopodia-like structures and in membrane fractions similar to lipid rafts. Down-regulation of CD147 levels in breast carcinoma cells causes corresponding changes in MT1-MMP expression, invasion, and invadopodia formation and activity. Cell sorting for high/low CD147 surface expression in breast cancer cells revealed that $CD147^{hi}$ cells were more invasive, formed more invadopodia, and were more complex intracellularly than $CD147^{low}$ cells. **Conclusions**: These findings indicate that CD147 regulates invadopodia formation and activity, most likely via assembly of MT1-MMP-containing complexes within lipid raft domains of the invadopodia. CD147 represents a novel cell surface target for combating cancer progression as it is enriched on the surfaces of numerous cancer types.

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UPEC Regulation of Chaperone Usher Pathway Pili

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Uropathogenic *E. coli* (UPEC) are the major cause of urinary tract infections (UTI), one of the most common infections afflicting females. Numerous bacterial factors have been shown to be important in UPEC UTIs, including type 1 pili. Pili assembled by the chaperone/usher pathway (CUP), such as type 1 pili, contain adhesins at their tips that are thought to play an important role in host-pathogen interactions. For example, FimH, the type 1 pilus tip adhesin, binds mannosylated glycoproteins on the luminal bladder surface to facilitate bacterial colonization and invasion of the bladder epithelium. Each sequenced UPEC strain has been found to encode a multitude of CUP pilus operons. Type 1, P and S pili have been associated with cystitis, pyelonephritis and neonatal meningitis and their receptor binding sites, function, expression and regulation have been well studied. However, little is known about the other CUP pili. In the P pilus model system, as pilus subunits enter the periplasm via the Sec translocase, they are bound by a periplasmic chaperone to form a stable chaperone/subunit complex that prevents subunit degradation. Pilus subunits are then polymerized through the outer membrane usher pore. We demonstrate that mutations in the genes encoding the chaperone or usher in the *fim* operon, which abolish pilus assembly, result in decreased expression of the operon. Simultaneously, these mutations that decrease expression of type 1 pili also increase expression of a subset of the other CUP pili, such as S and F17-like pili. Furthermore, UPEC double mutants lacking two CUP operons have increased expression of a subset of CUP pili. This work begins to elucidate the regulatory networks between the multiple extracellular appendages encoded by UPEC, and indicates a hierarchy of CUP pilus expression in a given environment. Further work examining the regulatory connections between the CUP pili will provide insight into how UPEC adapts to environments they colonize in order to persist in specific host, tissue and environmental niches.

Poster Abstracts

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Peripheral Nervous System Insulin Resistance

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Diabetes is the leading cause of peripheral neuropathy worldwide; however the etiology of diabetes-induced neuropathic complications remains unclear. Patients suffer from a variety of symptoms ranging from decreased proprioception and peripheral insensitivity, to allodynia and chronic nerve pain; collectively these symptoms can lead to foot ulcers and possibly amputation. The current research investigating the pathogenesis of diabetic neuropathy is predominantly focused on neuronal injury associated with the sequelae of hyperglycemia. However, the loss of neuronal insulin support has also been established as a crucial factor contributing to diabetic neuropathy. While decreased peripheral nervous system (PNS) insulin signaling is plausible in Type 1 diabetic (insulinopenic) models due to the paucity of insulin, abnormal insulin sensitivity in PNS neurons in Type 2 (hyperinsulinemic) models has not been explored. The purpose of this study is to characterize PNS insulin signaling and determine if insulin-resistant mice display alterations in activation of the insulin signaling cascade. Both *in vitro* and *in vivo* methods were employed to investigate PNS insulin signaling in insulin-resistant *ob/ob* mice. Insulin signaling in dorsal root ganglia (DRG) cultures from nondiabetic control and diabetic *ob/ob* mice was assessed with Western blots and neurite outgrowth assays. In addition, mice were also directly administered insulin through intrathecal injections and the responsiveness of insulin-signaling pathways was quantified in the DRG and sciatic nerve. Our results indicate that insulin-signaling abnormalities documented in other "insulin-sensitive" tissues (i.e. muscle, fat, liver) of *ob/ob* mice are also present in the PNS. A robust increase in insulin-induced Akt activation was detected in nondiabetic mice in both the sciatic nerve and DRG, however this response was blunted in both tissues from *ob/ob* mice. Furthermore, insulin supplementation resulted in a significant increase in neurite outgrowth in DRG cultures from nondiabetic mice, but not in DRG cultures from *ob/ob* mice. Finally, our results also indicate that upregulated JNK activation, IRS2 serine phosphorylation, and reduced insulin receptor expression could be contributory mechanisms of PNS insulin resistance. In conclusion, DRG neurons are susceptible to alterations in insulin signaling due to diabetes and these disruptions can inhibit the neurotrophic effects of insulin, contributing to the mechanisms that cause diabetic neuropathy.

76 (Cancelled)

A Randomized, Double-Blind Crossover Study of Sirtuin-Mediated Effects of an Intermittent Fasting Diet on Oxidative Stress and Mitochondrial Biogenesis

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Recent research suggests that metabolic dysregulation plays a central role in the aging process. Given this association, dietary interventions such as calorie restriction (CR) have been assessed for their anti-aging benefits. CR is hypothesized to induce transient levels of reactive oxygen species (ROS), which increase endogenous ROS protection pathways and mitochondrial biogenesis, preventing damage from subsequent ROS stresses. It is believed that sirtuin histone deacetylases help mediate these pathways. However, CR is impractical for healthy individuals to utilize on a longer-term basis, and it is not known if the benefits of CR persist in the absence of weight loss. Intermittent fasting (IF) has emerged as a more practical dietary fasting paradigm, which can promote comparable benefits when compared to CR diets. In this study, we examined whether an IF diet increases basal oxidative stress responses and mitochondrial biogenesis in a sirtuin-dependent manner. We tested this intervention with antioxidant supplementation to help elucidate the role that ROS may play in mediating these effects. We hypothesized that an intermittent fasting diet in healthy young volunteers would improve markers of cellular aging and that these beneficial effects would be abrogated by the supplementation of antioxidants. To test our hypothesis, we enrolled 17 patients into our cross-over placebo-controlled trial. Study participants went on the IF diet (alternating daily between fasting and feasting) for three weeks, then "washed-out" with a normal diet for three weeks, and then returned for another three weeks of the IF diet. During each IF period, participants supplemented their diet either with a placebo or with high-dose vitamin C and E in a randomized fashion. Blood was collected from each patient at the beginning and end of each IF period. Samples were analyzed for levels of ROS, as well as expression of antioxidant genes, sirtuins, and markers of mitochondrial biogenesis. The results of our study provide important insight into the mechanism and effects of IF as an anti-aging dietary intervention in the absence of weight loss. We anticipate that they will also inform the future design of well-tolerated dietary interventions associated with high rates of compliance when practiced on a long-term basis, which are effective in improving markers of aging.

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Therapeutic Potential of Astrocytes Derived From Glial Restricted Progenitors for Treatment of Spinal Cord Injury

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During CNS development astrocytes play a critical role in supporting neuronal survival and maturation, while in the adult they support synaptic transmission and structural integrity. However, astrocytes are also involved in the response to spinal cord injury (SCI) by forming a glial scar, which prevents axonal regrowth and thus hinders connectivity and functional recovery. Although there have been numerous studies highlighting the heterogeneity of astrocyte populations, the specific origin and properties of these populations remain mostly unknown. To examine these issues, we analyzed the differentiation of astrocytes from glial restricted progenitors (GRP) *in vitro* and their properties after engraftment into the injured spinal cord. GRP were obtained from rat embryonic spinal cord (E13.5) and differentiated into astrocytes using Bone Morphogenic Protein-4 (BMP4) and Ciliary Neurotrophic Factor

(CNTF), analyzed, and compared to GRP maintained in standard growth media containing basic Fibroblast Growth Factor. Morphological and immunochemical analysis with markers of cell maturation - A2B5 (GRP), GFAP (mature astrocytes), Nestin (neural progenitors), and Ki67 (proliferating cells) - was carried out to further define the properties of astrocyte populations. Cultures treated with BMP4 or CNTF produced short process $GFAP^+$ or elongate process $GFAP^+$ astrocytes, respectively. To evaluate the stability of the generated astrocytes, cultures were challenged with an alternate factor and demonstrated major phenotypic alterations, suggesting the presence of considerable plasticity. In addition, we have also analyzed human GRP (hGRP) isolated from fetal tissue (18-24 week) following the same differentiation protocols and observed similar results. Importantly, rat and human GRP and astrocytes transplanted into a dorsal column injury model of SCI, survived and supported axonal sprouting and regeneration into the injury site. These findings suggest that despite differences in astrocyte subtypes, these differentiation protocols did not produce terminal phenotypes. Furthermore, these findings also demonstrate that embryonic astrocytes remain capable of supporting axonal growth and may be used as a component of a therapeutic strategy for SCI.

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F-Spondin-Induced LDLR Activation in Alzheimer's Disease Pathology and Cognition

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Alzheimer's disease (AD) is the most prevalent form of dementia and its incidence is expected to significantly increase in the immediate future. Evidence has demonstrated that through activation of the low-density lipoprotein receptor (LDLR) family by Reelin, modulation of neurogenesis occurs in both development and adults. Additionally, signaling intermediates (GSK3, cdk5) in the LDLR activation cascade are kinases known to participate in hallmark AD pathologic pathways (Aβ plaques, *tau* tangles). Therefore, understanding the *in vivo* relationship between these two distinct processes may ultimately produce possible treatments or preventative mechanisms in combating AD. However, the gaps that currently exist in linking LDLR signaling intermediates in neurogenesis and AD pathologies must be bridged. This relationship will be explored through use of viral vector mediated over-expression of an LDLR stimulatory ligand (reelin homolog, F-spondin) or inhibitor (soluble secreted ApoE-R2 ligand binding domain). We report that stimulation of the LDLR pathway with the reelin homolog, F-spondin, significantly reduces amyloid-beta levels in wild-type mice. Additionally, when compared to the inhibitor, F-spondin-injected mice show significant reductions in plaque deposition in AD mice. Analysis of spatial memory using the Morris Water Maze demonstrates a significant improvement in platform acquisition and time spent in correct quadrant at 72 hours in wild-type mice injected with F-spondin compared to inhibitor and control groups. Using novel object recognition to assay episodic memory, F-spondin-injected mice spent significantly more time at the novel object in comparison to inhibitor and control groups. These data demonstrate pathologic and cognitive improvements in mice undergoing LDLR pathway stimulation by F-spondin over-expression.

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Sleep Disruption in Mice Induces Endoplasmic Reticulum Stress and Leptin Resistance in the Hypothalamus

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Introduction: Sleep Disruption (SD) is highly prevalent and may constitute an important contributing factor to excessive weight gain and the metabolic syndrome. Increased endoplasmic reticulum (ER) stress and activation of the unfolded protein response (UPR) leading to attenuation of leptin receptor signaling in the hypothalamus promotes obesogenic behaviors and metabolic dysfunction. We hypothesized that SD will induce ER stress, activate the UPR, and down-regulate leptin receptor signaling in the hypothalamus, thereby favoring increased adipose tissue accrual and metabolic dysfunction. **Methods:** Adult male C57/b6 mice were exposed to SD during the light period (from 7:00am to7:00pm) using a custom-designed apparatus based on automated tactile stimulation for 6 hours- 21 days along with matched controls (CO). Daily food intake and weight gain were monitored. Hypothalamic samples were harvested, and subjected to western blots using ATF6, eIF2α, p-eIF2α, p-ERK, HSP 70, HSP90, GRP78, FAT10, SOCS3, PTP1b, ObR, p-STAT3, STAT3, and β-Actin (as loading control), followed by quantitative and statistical analyses. Leptin receptor sensitivity was assessed following leptin or vehicle injection to both SD and CO. **Results**: After 3 days of SD, food intake was increased and sustained thereafter ($p<0.012$). SD induced ER stress in the hypothalamus from day 3 to day 7 SD (n=6), in 2 of the 3 major UPR pathways, with increased ATF6 ($p<0.04$) and p-eIF2α/ eIF2α ratios($p<0.04$), which were followed by increased immunoreactivity of GRP78 ($p<0.05$), HSP70 ($p<0.05$), HSP90 ($p<0.03$) and FAT10 ($p<0.06$) in SD mice. Leptin receptor (ObR) expression showed slight increases in expression, and in parallel p-STAT3/STAT3 decreased ($p<0.034$) suggesting reduced ObR receptor signaling. Although SOCS3 expression remained unaltered by SD, significant increases in PTP1b expression emerged ($p<0.04$) overtime, implicating up-regulation of PTP1b as a putative mechanism underling attenuation of leptin receptor signaling in SD. Furthermore, after leptin injection, reduced p-STAT3 responses emerged in SD mice. **Conclusion:** Sleep disruption in mice induces hyperphagic behaviors and reduced leptin signaling in the hypothalamus that appear to be mediated by ER stress and activation of the UPR. The increases in PTP1b expression further suggest this pathway as potentially promoting weight gain and metabolic dysfunction in the context of disrupted sleep. **Support**: DG is supported by National Institutes of Health grants HL-065270 and HL-086662.

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KLF15 Regulates Muscle Metabolism and is Essential for Exercise Adaptation

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In exercising mammals, two ancient neurohormonal systems have evolved to drive adaptive muscle metabolism and enhance performance – catecholamines and glucocorticoids (GCs). Catecholamines can regulate energy utilization in muscle via activation of the central transcriptional coactivator PGC-1a. In contrast, the parallel pathway by which GCs signal to such adaptive gene-networks is unknown and has been largely overshadowed by studies of GC-induced muscle atrophy. Here, we demonstrate that endogenous GCs signal via the exercise-inducible transcription factor KLF15 to control a substrate-flux/ergogenic program that is essential for muscle performance, and distinct from the atrogenic program. KLF15 is induced with exercise in both rodents and humans in a GC-dependent manner. KLF15 directly regulates a broad transcriptional program spanning all major segments of the skeletal muscle lipid-flux pathway (which fuels sustained endurance exercise) as well as several key enzymes involved in amino acid catabolism (which support hepatic gluconeogenesis and TCA cycle anaplerosis). Consequently, *Klf15*-deficient mice have abnormal substrate and energy flux, inappropriate reliance on carbohydrate fuels, exaggerated muscle fatigue, and impaired endurance exercise capacity. A central mechanism by which KLF15 induces its direct targets is through recruitment of the coactivator p300/CBP via its highly conserved acidic transactivation domain. Global expression profiling reveals that a substantial fraction of the GC-regulated muscle transcriptome is KLF15-dependent, with enrichment for genes involved in metabolic adaptation and exclusion of classical atrogenes. Accordingly, we demonstrate that KLF15 is required for the ergogenic effects of transient GC exposure, but dispensable for chronic GC-induced muscle atrophy. This study, which is the first to implicate the KLF gene family in muscle physiology and exercise adaptation, illustrates a fundamental neurohormonal mechanism by which animals exert coordinated control over lipid utilization, amino acid catabolism and glucose production during metabolic stress. Given the well-known health benefits of exercise and the intense interest in selective modulation of glucocorticoid receptor function, the ability of KLF15 to dissociate performance-enhancing from atrophic effects of GCs may have important therapeutic potential in metabolic and myopathic diseases.

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XIAP mono-ubiquitylates Groucho/TLE to Promote Canonical Wnt signaling

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A key event in Wnt signaling is conversion of TCF/Lef from a transcriptional repressor to an activator, yet how this switch occurs is not well understood. Here, we report an unanticipated role for X-linked Inhibitor of Apoptosis (XIAP) in regulating this critical Wnt signaling event that is independent of its anti-apoptotic function. We identified DIAP1 as a positive regulator of Wingless signaling in a *Drosophila* S2 cell-based RNAi screen. XIAP, its vertebrate homolog, is similarly required for Wnt signaling in cultured mammalian cells and in *Xenopus* embryos, indicating evolutionary conservation of function. Upon Wnt pathway activation, XIAP is recruited to TCF/Lef where it mono-ubiquitylates Groucho(Gro)/TLE. This modification decreases affinity of Gro/TLE for TCF/Lef. Our data reveal a transcriptional switch involving XIAP-mediated ubiquitylation of Gro/TLE that facilitates its removal from TCF/Lef, thus allowing β-catenin-TCF/Lef complex assembly and initiation of a Wnt-specific transcriptional program.

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Characterisation of Apoptotic Responses in a Pituitary-Derived Cell Line

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Background: Pituitary adenomas are the most common tumours of the central nervous system, accounting for 10% of primary intracranial neoplasia. We have recently identified a novel pituitary derived gene, the expression of which is lost in the majority (79%) of primary human pituitary adenomas. Expression of this gene was found to significantly augment drug-induced apoptotic stimuli, and on this basis it was assigned the name Pituitary Tumour Apoptosis Gene (PTAG). PTAG was shown to be under-expressed in most pituitary adenomas through methylation of its promoter region leading to gene silencing and oncogenic transformation. While genetic alteration is usually irreversible, epigenetic methylation is potentially reversible and provides the possibility for future treatment interventions. **Objectives**: This study aims to determine if PTAG expression is regulated by bromocriptine-induced apoptotic stimuli, and to investigate the relationship between PTAG expression and the activity of its promoter region. **Methods**: Bromocriptine-induced apoptotic response was assessed using Hoechst staining. RNA extraction was performed on pituitary tumour cells treated or untreated with bromocriptine up to 48 hours of treatment, and PTAG expression was determined employing quantitative Real Time-PCR (qRT-PCR). PTAG promoter activity was examined using transient transfection of PTAG promoter-reporter (luciferase) constructs and the *Renilla* construct, and reporter activity was determined through chemiluminescence. **Results**: Treatment with increasing doses of bromocriptine induced a proportional increase in apoptotic cells. Maximal PTAG promoter activity was found to result from the 5′ flanking region between -170 and -135. This activity did not increase upon exposure to bromocriptine-induced apoptotic stimuli. **Conclusion**: Bromocriptine-induced apoptosis demonstrates a dose-response relationship, where the percentage of apoptotic cells in pituitary adenoma increases according to the dose of treatment. Mapping of the PTAG promoter sequence showed that the minimal PTAG promoter is extremely compact, with the maximal transcriptional activity produced from a 35bp sequence situated between nucleotides -170 and -135 upstream of

the transcription start point. Further sequencing analysis revealed the basal promoter to harbour transcription factor binding sites that potentially determine PTAG expression.

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Polyphosphate in Bacterial Starvation Signaling and Cell Cycle Regulation

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All bacteria, including pathogens and commensals, undergo a regulated cell cycle by which growth and cell division occur. Studies of the model bacterium *Caulobacter crescentus* have facilitated unique insight into prokaryotic cell cycle regulation. *C. crescentus* differentiates from a motile, foraging swarmer cell into sessile, replication-competent stalked cell. This developmental transition is inhibited by nutrient deprivation to favor the motile swarmer state. We identify a novel cell-cycle regulatory molecule, inorganic polyphosphate (polyP), which inhibits the swarmer-to-stalked transition in both rich and glucose-exhausted media, thereby increasing the proportion of swarmer cells in mixed culture. PolyP accumulates in *C. crescentus* cells upon depletion of available carbon. Under these conditions, swarmer cells lacking the ability to synthesize polyphosphate improperly initiate chromosome replication, proteolyze the replication inhibitor CtrA, localize the cell-fate determinant DivJ, and develop polar stalks. Moreover, overexpression of the polyP biosynthetic enzyme is sufficient to slow the cell cycle, even in rich media. These results provide evidence that polyP acts as a cell-type specific developmental regulator in our model bacterium. Furthermore, polyP exhibits a distinct subcellular localization pattern, such that polyP stores are divided between mother and nascent daughter cells prior to cell division. Without polyP, cells display markedly reduced survival under carbon starvation. Since polyP is known to be required for survival and virulence of other bacteria, including many mammalian pathogens, we believe future studies may shed light on the role of this substance in a widely conserved bacterial stress response pathway.

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Depression as a Disease of Modernity: Explanations for Increasing Prevalence

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There has been much speculation about modern environments causing an epidemic of depression. This review aims to (1) determine whether depression rates have increased and (2) review evidence for possible explanations. While available data indicate rising prevalence and an increased lifetime risk for younger cohorts, strong conclusions cannot be drawn due to conflicting results and methodological flaws. There are numerous potential explanations for changing rates of depression. Cross-cultural studies can be useful for identifying likely culprits. General and specific characteristics of modernization correlate with higher risk. The correlation between a country's GDP per capita, as quantitative measure of modernization, and lifetime risk of a mood disorder trends toward significance (r=0.46, p=0.06). Mental and physical well-being are intimately related. The growing burden of chronic diseases, which arise from an evolutionary mismatch between past human environments and modern-day living, may be central to rising rates of depression. These obesity-related diseases are endemic to industrialized nations. So-called "diseases of modernity" share many behavioral risk factors and co-morbidity with depression, as well a similar physiologic profile of metabolic and inflammatory dysregulation. Declining social capital and greater inequality and loneliness are candidate mediators of a depressiogenic social milieu. Between modern-industrialized nations, income inequality correlates with lifetime risk of a mood disorder (r=0.72, p=0.03). Modern populations are increasingly overfed, malnourished, sedentary, sunlight-deficient, sleep-deprived, and socially-isolated. These changes in lifestyle each contribute to poor physical health and affect the incidence and treatment of depression. This theoretical framework will hopefully aid in the understanding, prevention, and treatment of depression and other diseases of modernity at the clinical and population level.

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Analysis of Microvessels Within Carotid Artery to Determine Plaque Vulnerability

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Background: A challenge facing surgeons is selection of patients for carotid endarterectomy. While some patients with carotid atherosclerosis develop unstable plaque and subsequent stroke, others form more stable plaque and are asymptomatic. Previous work implicates intimal microvessels as key contributors to plaque instability, and identifies the angiogenic growth factor HGF as a possible biomarker. Identifying differences between mature and leaky microvessels may help to predict risk of plaque rupture and guide surgical intervention to patients who will most benefit. **Method:** Endarterectomy specimens were taken from symptomatic and asymptomatic patients; microvessel morphology was interrogated using immunofluorescence. Post-mortem human carotid artery specimens were stained for mature and leaky microvessels, dissected out with either fine-needle or laser-capture micro-dissection for RNA extraction. Patient serum was analysed for the concentration of 27 cytokines. Ten patients with carotid disease and ten healthy controls were recruited. **Results:** Mature and leaky microvessels were seen to co-exist within carotid artery adventitia, displaying stable and unstable morphology respectively. Laser-capture of microvessels was achieved enabling isolation of selected vascular cells from adventitial tissue. Levels of the pro-inflammatory cytokines, interleukin (IL)-6 and IL-15 were significantly elevated in patients compared to controls ($p<0.05$) The chemotactic cytokines eotaxin and MCP-1 were also elevated ($p<0.01$), together with the anti-inflammatory cytokine interleukin-1 receptor antagonist ($p>0.05$). Symptomatic patients were found to have significantly reduced circulating levels of platelet-derived growth factor. IL-12 and IL-17 levels were higher in symptomatic compared to asymptomatic patients ($p<0.05$). **Conclusion:** These data support an association between circulating inflammatory cytokines, growth factors and symptomatic carotid disease.

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Postnatal Developmental Alterations in Axon Initial Segment Innervation in Monkey Prefrontal Cortex

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Background: Schizophrenia is a devastating neuropsychiatric illness that is well recognized for the presence of delusions and hallucinations; however, deficits in cognitive control are considered core features of the illness and are the best predictor of functional recovery. Substantial evidence suggests that schizophrenia is a neurodevelopmental disorder with altered cortical GABA neurotransmission. Parvalbumin-containing (PV+) chandelier cells provide arrays of GABA terminals (cartridges) that exclusively innervate pyramidal cell axon initial segments (AISs), the principal site of action potential generation. In the dorsolateral prefrontal cortex (DLPFC), the density of cartridges immunoreactive for certain proteins is altered in schizophrenia and changes during postnatal development in monkeys. However, methods used were unable to distinguish between differences in terminal number per cartridge or protein levels per cartridge terminal. To discriminate between these possibilities, we determined the postnatal developmental trajectories of cartridge terminal number and PV protein level per cartridge terminal in macaque monkey DLPFC, a brain region that is critical for cognitive control. **Methods:** DLPFC tissue from macaques (1, 3, 18, 45, and 156 months old; n=3 animals/age group) was used for confocal immunofluorescence microscopy. Cartridge terminals were labeled with the vesicular GABA transporter (vGAT) and PV; pyramidal cell bodies with NeuN; and AISs with ankyrin-G (AnkG) and $GABA_A$ receptor γ2 (γ2). Terminal number, density, relative fluorescence intensity and AIS length were measured in pre- and postsynaptic overlapping vGAT+/PV+/ γ2+ puncta apposed to AnkG+ AISs emanating from NeuN+ somata. **Results:** The frequency of highly innervated AISs and AIS length decreased significantly ($p<0.01$) during postnatal development in the DLPFC, without a change in innervation density. Fluorescence intensities of terminals containing overlapping vGAT/PV puncta and postsynaptic clusters of γ2 at AISs increased. **Conclusions:** Maturational changes in cartridge terminals and AISs may include pruning of cartridge terminal number along with protein level increases in cartridge terminals and postsynaptic AISs. These findings may inform the timing and nature of alterations in chandelier cell innervation of pyramidal cells in schizophrenia.

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Identification of C-Myc Inhibitor, the Triterpenoid Celastrol

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c-Myc is a bHLH-ZIP transcription factor that is deregulated in a variety of human cancers.More specifically, c-Myc heterodimerizes with another bHLH-ZIP partner protein, Max, to bind to specific sequences ("E-Boxes": CAC/TGTG) located near the transcriptional start sites of its numerous target genes. Because c-Myc's highly variable downstream effects are difficult to attack individually, directly inhibiting the association between c-Myc and Max association by interfering with their bHLH-ZIP-mediated heterodimerization has emerged as an attractive therapeutic strategy. Thus far, high throughput screens of chemical libraries have identified a number of small molecules that specifically inhibit the c-Myc-Max association, However, these synthetic compounds and their analogs are generally of low potency and therefore have limited clinical utility. Here we report identification of celastrol, a naturally occurring triterpenoid, as a potent c-Myc inhibitor. We sought to further assess celastrol's ability to inhibit c-Myc-Max interaction and cell growth, and determine its structure activity relationship. Surface plasmon resonance and electrophoretic mobility shift studies revealed that celastrol specifically binds to c-Myc and prevents both its interaction with Max, and subsequent DNA binding with a Kd in the low micromolar range. Co-immunoprecipitation studies confirmed that the dose-dependent disruption of c-Myc-Max heterodimers by celastrol could also be achieved in c-Myc-overexpressing human HL60 promyelocytic leukemia cells. Moreover, in vitro data demonstrated that celastrol significantly inhibited both HL60 and Burkitt's lymphoma cell growth with IC50s in the nanomolar range. However, modifications to the C-28 carboxylic acid group of celastrol eliminated c-Myc binding, suggesting that celastrol binds to c-Myc via this functional group. This structure activity relationship may provide a basis for the development of more pharmacologically suitable analogs. Together these results suggest that celastrol is among the most potent Myc-Max disruptors yet identified, and suggest a mechanism of action. Celstrol thus represents a promising new agent with which to target c-Myc and provides a new structural chemical platform for further pharmaceutical development.

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Dynamics and Regulation of the ACF Chromatin Remodeling Complex at the Single-Molecule Level

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Packaging genomic DNA into chromatin is necessary to fit the nearly two meters of human DNA into each cell nucleus. However, this extreme degree of condensation occludes many regulatory elements. Thus, eukaryotic cells depend on a dynamic balance between genome compaction and access facilitated by histone modifying enzymes and chromatin remodeling complexes. Chromatin remodelers are ATP-dependent molecular motors that assemble, disassemble, translocate, and modify the composition of nucleosomes. Importantly, defects in the chromatin remodeling machinery underlie many cancers and multisystem developmental disorders. Uncovering the mechanisms of chromatin remodelers is a significant challenge because they catalyze reactions that are both biochemical and mechanical in nature and too complex to fully understand with conventional ensemble methods. Probing the dynamics of individual chromatin remodelers in real-time with single-molecule techniques can help fill this knowledge gap.
Human ATP-dependent chromatin assembly and remodeling factor (ACF) is an important member of the ISWI family of

chromatin remodeling complexes and catalyzes both nucleosome assembly and repositioning. In particular, dimeric ACF centers a single nucleosome on a DNA fragment and evenly spaces out multiple nucleosomes in an array, a hallmark of repressive heterochromatin. We developed a single-molecule fluorescence resonance energy transfer (smFRET) assay to study the dynamics of chromatin remodeling in real-time. We discovered that ACF-mediated nucleosome sliding features well-defined kinetic pauses after approximately 7 or 3-4 bps of translocation, dividing the remodeling process into alternating pause phases and translocation phases that correspond to two different types of ATP-dependent processes: (Step 1) pause phase - initiation event that involves loosening histone-DNA contacts and forming a template-committed intermediate, and (Step 2) translocation phase - propagation of loosened DNA across the histone octamer resulting in nucleosome repositioning. The linker DNA length is an important regulatory factor for ACF remodeling. We found that shortening the linker DNA reduces the rate of Step 1 but has no effect on Step 2, suggesting that the linker DNA plays a critical role in the formation and/or stability of the template-committed complex but not in the propagation of loosened DNA across the histone octamer.

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3D Image Guided Reconstructive Microsurgery Using Ultra High Speed Fourier Domain Optical Coherence Tomography

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Background: Micro-vascular anastomosis is an integral component of free tissue transfer and reconstructive transplantation. Even in the era of highly magnified images from optical microscopes, this technique still requires the highest level of skill especially for small vessels (Diameter < 1.0mm). We designed an innovative surgical imaging modality that can provide depth perception and real time 3-D intraoperative guidance for micro-vascular surgery. **Methods:** We developed an ultra-fast 3D optical coherence tomography (OCT) imaging system that provides real-time intraoperative image of the surgical site during the micro-surgical procedures. The system is based on a Fourier-Domain OCT (FD-OCT) integrated with a CPU-GPU heterogeneous computing architecture, capable of providing real-time 3-D video image of surgical site and tool tips. We optimized FD-OCT settings to visualize rat femoral artery (Diameter < 0.8 mm). **Results:** Utilizing ultra fast real-time intraoperative imaging, we were able to visualize the cut end of the vessel lumen in six different views. Thus we could precisely place 11-0 sutures through the vessel wall without the use of optical microscope. In addition, we could obtain an optimized view of the conventional microsurgical instruments, needle and thread in real time. Ultra fast imaging capture and display was sufficient to maneuver the instrument with advanced precision. **Conclusion:** 3D-OCT assistance during critical portions of microvascular anastomosis can provide better precision and can minimize human error. In addition, our laser system can determine the diameter of vessel post anastomosis and can also determine the blood flow-using Doppler OCT. These additional properties can promptly diagnose diminished flow or vessel narrowing at anastomosis site and hence the dreading complication of flap necrosis can potentially be avoided.

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$CD8^+$ T Cell Exhaustion during Chronic Viral Infection is Regulated Downstream of the Virus-Specific T Cell Receptor

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$CD8^+$ T cell exhaustion during chronic viral infection is characterized by the progressive loss of anti-viral $CD8^+$ T cell numbers and function, which compromises viral control and increases disease pathology and risk of transmission. Like exhaustion, tolerance in response to encounter with tumor/self-antigen is also characterized by the loss of reactive T cells and impaired function in the few that remain. Our prior work demonstrated that immunization of tolerant dual-T cell receptor (dual-TCR) $CD8^+$ T cells through a T cell receptor (TCR) specific for foreign antigen restored proliferative capacity and effector function toward tumor despite persistent tolerizing signals through the self-reactive TCR. These data revealed that tolerance is regulated at the self-reactive TCR rather than at a more global cellular level. Our current work examines whether the functional defects observed in exhausted anti-viral $CD8^+$ T cells are regulated by the virus-specific TCR or by more global cellular changes. Here, we show that dual-TCR $CD8^+$ T cells acquire phenotypic and functional characteristics of exhaustion in the context of a chronic lymphocytic choriomeningitis virus (LCMV Clone 13) infection. We further demonstrate that immunization of exhausted dual-TCR $CD8^+$ T cells through a second TCR, which does not engage viral antigen, fails to elicit expansion of dual-TCR T cells or restore the cytokine defect that they incur as a result of persistent antigen encounter. These results imply that T cell vaccination via a secondary TCR alone is insufficient to restore exhausted $CD8^+$ T cell responses, and that unlike tolerance, exhaustion is governed by cellular defects downstream of the virus-specific TCR. These novel observations establish that exhaustion and tolerance are distinctly regulated states of T cell dysfunction, and could inform the design of future therapeutic interventions.

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RAB26, A Transcriptional Target of MIST1, Regulates Lysosomal Trafficking in Exocrine Secretory Cells

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Exocrine cells like pancreatic acinar and zymogenic chief cells have a highly developed secretory architecture that is rapidly established upon maturation. The evolutionarily conserved transcription factor MIST1 governs normal apical secretory vesicle organization in these cells. Here we show that the direct MIST1 transcriptional

target RAB26 is a novel lysosome associated protein whose forced expression repositions lysosomes, in a GTPase-dependent manner, from the cell periphery to a perinuclear region. In mouse exocrine cells that lack RAB26 because they are null for *Mist1*, lysosomes are abnormally distributed. Normally, lysosomes cluster in a centralized basal compartment away from secretory granules, whereas in *Mist1−/−* exocrine cells they accumulate apically and degrade secretory vesicles. Thus, we propose MIST1 promotes normal accumulation of secretory granules through blocking their targeting by the cellular degradation/recycling machinery. The results illustrate how a transcription factor can regulate cell architecture and have implications for disease processes like acute pancreatitis where MIST1 is lost, and secretory vesicles are targeted by lysosomal enzymes.

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Transoral Surgery with Novel Highly Flexible Robot

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Introduction: Transoral robotic surgery (TORS) offers patients minimally invasive surgical procedures as an alternative to traditional open surgery. Currently, virtually all TORS procedures are performed with the da Vinci Surgical System. The da Vinci system's design has a bulky nature and utilizes long, rigid line of site instruments. These characteristics can make access to certain sites within the laryngopharynx and instrument maneuvering at those sites extremely difficult, especially within the narrow laryngopharynx. A novel highly-flexible robot provides unhampered access to all sites within the laryngopharynx for surgical therapies, and we demonstrate the utility of the flexible robot for performing a variety of procedures common to head and neck oncology. **Methods:** Controlled by the surgeon using an adaptive joystick controller, the highly-flexible robot was driven to several anatomical sites within the laryngopharyngeal complex. End-effectors were inserted through the robot's tooling ports to retract, cauterize and remove tissue in various procedures including epiglottectomy, base of tongue resection and vocal cord excision. Time to tissue exposure was noted for each procedure. Each epiglottectomy was timed to determine if operation time was comparable to current TORS procedures. **Results:** Epiglottectomy, base of tongue resection, and vocal cord excision were successfully performed in each attempt without suspension laryngoscopy. Individual surgeons improved robot-driving times significantly between first and second attempts ($p=0.037$), representing a shallow learning curve. Epiglottecomies were performed in an average time of 42 minutes ($N=5$, $\sigma = 28$ minutes). **Conclusions:** The highly flexible robot may prove to be an invaluable surgical tool in head and neck oncology. Compared to other surgical robots, the highly flexible robot offers improved maneuverability and triangulation for procedures in the endolarynx.

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Dexamethasone Leads to Memory Deficits and Altered Tau Phosphorylation in a Transgenic Mouse Model of Alzheimer's Disease

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Background: Several studies have linked altered glucocorticoid levels with Alzheimer's disease (AD) pathogenesis, however, the mechanism remains to be fully elucidated. **Method:** In the current work, we administered the glucocorticoid dexamethasone (5mg/kg) daily to Tg2576 mice, a transgenic animal model of AD, for 28 d and investigated its effect on memory, amyloid beta (Aβ) and tau. **Results:** At the end of the treatment period we observed that mice receiving dexamethasone (dex) had a significant impairment in the fear conditioning paradigm compared with controls, exhibiting diminished percentage freezing time in the cued recall portion (control: $16.0 \pm 2.6\%$, dex: $5.9 \pm 2.5\%$; $p<0.02$). Dexamethasone-treated animals showed a significant increase in the amount of brain soluble Aβ 1-40 levels relative to control (control: $100.0 \pm 10.39\%$, dex: $166.0 \pm 17.55\%$; $p<0.004$), but no alteration in the steady state levels of its precursor protein, APP, or in the major protease enzymes involved in its metabolism (i.e., ADAM-10, BACE-1 or the γ-secretase complex). While total tau protein levels in the brain were unaltered between the two groups, we found that compared to control animals, dexamethasone treatment significantly reduced tau phosphorylation at the ser202/thr205 (control: $100.0 \pm 23.0\%$, dex: $26.9 \pm 8.4\%$; $p<0.001$) and ser396 residues (control: 100.0 + 16.2 %, dex: 53.7 ± 8.0 %; $p<0.02$). Alterations in tau phosphorylation were mediated by changes in glycogen synthase kinase-3beta (GSK3β), with dexamethasone treatment significantly decreasing its steady-state protein level (control: $100.0 \pm 10.0\%$, dex: $55.7 \pm 6.7\%$; $p<0.004$) and activity (control: $1.4\times10^7 \pm 5.9\times10^5$ arbitrary units [AU], dex: $9.0\times10^6 \pm 1.1\times10^6$ AU; $p<0.005$). Finally, we observed a direct correlation between memory impairments and tau phosphorylation levels. **Conclusion:** Our study highlights the significant role that glucocorticoids play in exacerbating AD-like cognitive impairments via alteration of tau protein phosphorylation state.

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Redox-State-Based Screening for Drugs That Have the Ability to Treat Endoplasmic Reticulum Diseases

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Recent evidence suggests that the endoplasmic reticulum (ER) plays a crucial role in making life-or-death decisions of mammalian cells. Defects in ER homeostasis direct most cells from a pro-survival to a pro-death state. Two major mechanisms maintaining ER homeostasis are redox regulation and the unfolded protein response (UPR). Previous studies have shown that shifting the ER from a reducing to an oxidizing environment may be useful in ameliorating ER stress. Using a real time monitoring system

of ER redox status we established, we found that β cell stress inducers such as palmitate, inflammatory cytokines, and chronic high glucose shift the ER towards a reducing environment, thus highlighting the importance of identifying compounds that can alter the ER redox state. Here, we report on a redox-state-based screening method to identify drugs that have the ability to shift the ER from a reducing toward an oxidizing environment under ER stress conditions. We identified several clinically used drugs never before linked to redox regulation or the UPR, including pioglitazone and apomorphine. We further show that treatments with these drugs confer protection against ER stress-mediated cell death in a cell model of ER disease, Wolfram syndrome and in primary human islets. Redox-state-based screening may provide a useful framework for understanding and developing novel therapeutics for diseases caused by ER dysfunction such as diabetes, neurodegeneration, and Wolfram syndrome.

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Fluctuations in the Prevalence of Heparin Induced Thrombocytopenia Antibodies during the Period 2004-2012. Relevance to Heparin Contaminant Crisis in 2007-2008.

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In conjunction with an ongoing program to investigate the prevalence of HIT antibodies (HIT Ab) in End Stage Renal Disease (ESRD), blood samples were collected from patients (n=53-119) on maintenance dialysis and retrospectively analyzed for the presence of these antibodies during the period February-January 2004-2012. Specified cut off levels were used to determine the positive and negative results. An ELISA method (GTI. Brookfield, WI) was used to quantitate the HIT antibodies. The prevalence of the HIT Ab during the period 2004-2012 was < 15% with the exception of 2007 when it was 35% and in 2008 it was 30%. A decreased prevalence was noted during the period 2009-2011 (13-15%). In the most recent blood samples (n=119) analyzed in January 2012, there was an even lower prevalence of HIT antibodies (<10%). The antibody titers as quantified by optical density measurement were relatively higher during the years 2007 and 2008. Moreover, the subtyping of these antibodies in 2007 and 2008 showed a higher proportion of IgG subtype. Interestingly, the platelet counts did not differ in the different groups. Similar trends were noted in the prevalence of HIT antibodies when other methods to detect HIT antibodies were used. The oversulfated glycosaminoglycans such as oversulfated chondroitin sulfate and other non-heparin GAG derivatives (hypersulfated dermatan sulfate) are most likely to contribute to this increased prevalence and seroconversion of these HIT antibodies. These observations suggest that heparin contaminants such as the oversulfated non-heparin glycosaminoglycans have contributed to the higher prevalence of HIT antibodies during the period 2007-2008. Furthermore, because of the stringent FDA requirements for quality assurance of heparin batches used for clinical purposes, the observed prevalence after 2009 is within the expected range of <15% (<10% in 2012).

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Modeling Actin Cytoskeleton Alignment under Cyclic Mechanical Loading in Vasculature

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Background: Mechanics affects all aspects of human body functioning from blood flow to alveolar expansion to traumatic brain injury. Here, we describe a coarse-grained Monte Carlo model of the actin filament cytoskeleton in a cell undergoing uniaxial cyclic stretch coded in MATLAB. We demonstrate a gradual alignment of the actin filaments perpendicular to the direction of stretch that reflects a similar alignment of endothelial cells and fibroblasts, suggesting a biomechanical similarity that our model supports. We support our model with experimental data showing gradual alignment of fibroblasts perpendicular to 1 Hz cyclic stretch. **Simulation setup:** We used a two-dimensional circular solution space of prescribed radius that is considered fixed to an underlying substrate at pre-determined perimeter nodes, representative of focal adhesions fixing a cell on a substrate. A number of free nodes representative of cross-linking proteins are created in the circle's interior. Filaments representative of actin filaments are randomly generated between nodes. Stretching is simulated by displacing the perimeter nodes, creating imbalanced forces on the free interior nodes that are iteratively relaxed until force equilibrium is achieved. Filaments remaining at high stress are probabilistically removed and replacements are generated to complete a stretch cycle. **Experiment setup:** NIH 3T3 fibroblasts were washed and seeded at a concentration of 1000 cells/cm^2 on polydimethylsiloxane (PDMS) substrates pre-coated with fibronectin in standard media with supplements. An air pressurization system for generating cyclic uniaxial stretch on cells adhered to the PDMS membrane was created using a custom pressure modulation mechanism. Following mechanical stimulation, the cells were stained for F-actin, imaged using an inverted optical microscope, and analyzed via ImageJ software. **Results and Discussion:** Our model demonstrates a clear affinity for vertical alignment relative to stretch direction as cycle number increases. In addition, a trend towards decreased filament stresses in both tensile and compressive directions is observed, suggesting a mechanistic response where filaments align to minimize their energy. The orientation of NIH 3T3 fibroblast actin filaments exposed to 1 Hz cyclic uniaxial stretch after 24 hours qualitatively follows the same trend as our simulations.

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HSV-1 and HSV-2 Have Different Receptor Requirements for Infection of the Murine Cornea

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Herpes Simplex Virus (HSV) is a frequent cause of corneal disease and the most common cause of infectious blindness in the U.S. Infection is initiated by the viral glycoprotein gD binding to a

receptor on host cells and triggering the fusion of the viral envelope with the host membrane via a poorly understood mechanism. Two host receptors have proven important in *in vivo* murine models of HSV infection. In the absence of both herpesvirus entry mediator (HVEM) and nectin-1, HSV-2 is unable to infect mice by either intravaginal or direct cranial inoculation. In HVEM knockout (KO) mice, HSV-2 infection is indistinguishable from disease observed in wild-type (WT) mice. When only nectin-1 is removed the infection is attenuated particularly in spread within the nervous system. Recent studies in our lab demonstrated that when either HVEM or nectin-1 is absent HSV-1 infection is attenuated in mice inoculated via the cornea. While both HVEM KO and nectin-1 KO mice can be infected with HSV-1 via the cornea, they fail to develop extraocular lesions, signs of neurological infection, and viral titers collected from eye swabs, periocular skin, trigeminal ganglia, and brains are lower than those of their WT counterparts. These findings are in contrast to the aforementioned HSV-2 studies that found HVEM to be dispensable for infection except in the absence of nectin-1. Our current studies focus on understanding the contrasting results between HSV-1 and HSV-2. Here we explore both HSV-1 and HSV-2 infection of the murine cornea using both WT and HVEM KO mice. Three different strains of both HSV-1 and HSV-2 were used to infect the mice via corneal scarification. The mice were monitored for symptoms of infection and viral titers from eye swabs and relevant tissues were collected and analyzed. Interestingly, all HSV-1 strains appear to be attenuated in HVEM KO mice while HSV-2 strains cause similar disease in WT and HVEM KO mice when inoculated via the cornea. These findings suggest that the interaction between HSV and its receptor, HVEM, is more complex and nuanced than previously thought.

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Stenotrophomonas Maltophilia Murine Pneumonia and the Study of Potential Virulence Factors

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Stenotrophomonas maltophilia (*Sm*) is a Gram-negative bacterium that is an emerging opportunistic pathogen commonly associated with hospital-acquired pneumonia and blood stream infections as well as infecting those with cystic fibrosis. Very little about the basic biology of this bacterium and its pathogenesis of infections is known. To gain further understanding of this organism, a murine model of *Sm* pneumonia was sought. Using an intranasal inoculation of A/J mice, strain K279a (i.e., the sequenced strain of *Sm*) is capable of replicating in the lungs of mice, displaying as much as 10-fold increase in CFU in the first 8 hours. Subsequently, bacterial numbers declined over the next 12-48 hours. Bacterial replication in the lungs was accompanied by elevations in pro-inflammatory cytokines and promoted resistance to subsequent challenge. *Sm* may possess many factors that influence the outcome of an infection. The study of two such factors has begun to be studied; type IV pili and type II protein secretion. These two systems have shown to be virulence factors in other bacterial species, and thus have been targeted. By making mutants in type IV pilus and type II secretion systems, in vitro and in vivo phenotypes have been studied.

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The Role of Prostatic Acid Phosphatase in Peripheral Neuropathy Associated With Type 1 Diabetes

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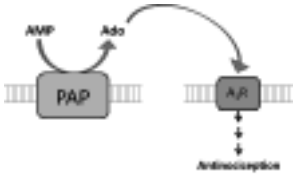
Diabetes affects more than 220 million people worldwide, and up to 50% suffer from peripheral neuropathy. Of those patients with neuropathy, approximately 15-30% experience debilitating pain. Recent evidence suggests the enzyme prostatic acid phosphatase (PAP) plays an important role in regulating nociception; however, the biology of PAP associated with pain sensation in diabetic neuropathy (DN) has not been investigated. PAP reduces pain by cleaving adenosine monophosphate (AMP) to adenosine, a well-known analgesic small molecule that signals through the A_1 adenosine receptor (A_1R). Our goal is to evaluate the effect of diabetes on this signaling pathway using a murine model of Type 1 diabetes. In this study, male A/J mice were given intraperitoneal streptozocin to induce Type 1 diabetes. Blood glucose levels, weight and behavior were monitored weekly for seven weeks. Diabetic mice had significantly elevated blood glucose levels ($p<0.0001$) throughout the study, and their weight decreased by almost 19% whereas nondiabetic mice gained weight as expected.



Diabetic mice displayed significant mechanical allodynia beginning four weeks post-diabetes induction ($p<0.05$) that continued throughout the study. At the conclusion of the study, lumbar spinal cord (SC) and dorsal root ganglia (DRG) were harvested and analyzed for PAP expression via immunohistochemistry and Western blot analysis. PAP expression was observed primarily on small-diameter non-peptidergic neurons in the DRG and in nerve terminals in lamina II of the dorsal SC; PAP expression overlapped extensively with the nonpeptidergic marker IB4. Little to no expression was observed on $CGRP^+$ peptidergic neurons in the SC or DRG. Protein expression levels of PAP were significantly decreased in the DRG of diabetic mice compared to their nondiabetic counterparts ($p<0.05$). However, there was no significant difference in PAP expression in the SC of diabetic mice compared to nondiabetic mice. A_1R expression does not appear to be altered in diabetes (data not shown). Collectively, these studies suggest that PAP levels may be altered in diabetic sensory neurons and these changes may modify nociceptive symptoms associated with diabetic neuropathy.

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Tcf19 Is a Novel Transcription Factor Necessary for Pancreatic Beta Cell Proliferation and Survival

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Diabetes is a disease caused by reduced pancreatic beta cell mass. Understanding the regulation of adaptive proliferation

and prevention of apoptosis in the beta cell will be necessary to develop new therapeutics to prevent and treat diabetes. We have identified Tcf19 as key factor in the regulation of beta cell proliferation and survival. Tcf19 is a putative transcription factor containing a forkhead-associated (FHA) domain. It is largely uncharacterized, but is expressed during cell division, beginning at G1/S phase. In a mouse model of obesity-associated diabetes, we identified *Tcf19* within a group of cell cycle genes whose expression correlates with adaptive islet proliferation in response to obesity. We went on to show that *Tcf19* is most highly expressed in the mouse islet across many other tissues. In addition, Tcf19 localizes to the nucleus of the beta cell, supporting a role in beta cell transcriptional regulation. Further, we see a trend toward increased expression of *TCF19* in obese human islet when compared with islets from non-obese individuals. We then directly examined the role of Tcf19 in beta cell growth. After siRNA-mediated knockdown of *Tcf19* in a transformed beta cell line, we found a significant reduction in the number of viable cells (n=5, $p<0.05$) and a 45% decrease in cell proliferation as measured by ^{3}H-thymidine incorporation (n=3, $p<0.01$). *Tcf19* knockdown also led to a decrease in expression of many key cell cycle regulatory genes, including *A-*, *B-*, and *E-type cyclins*, *Mki67*, *Pbk, Cdca3,* and *Bub1* (17-53%, n=3, $p<0.05$). After *Tcf19* knockdown, cells fail to progress normally beyond the G1/S checkpoint. We saw a concomitant increase in apoptosis with *Tcf19* knockdown, both at baseline (1.57-fold) and when treated with thapsigargin, which induces ER-stress mediated apoptosis (2.57-fold, n=3, $p<0.05$). As a potential mechanism to explain the increased apoptosis, we identified a 37% decrease in expression of *P58^{IPK}*, which is protective in mediating the unfolded protein response during ER-stress. In summary, *Tcf19* appears to be a key regulator of beta cell survival and proliferation and may be important in the adaptive expansion of beta cell mass to compensate for insulin resistance and obesity.

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Feeder Free Generation of Natural Killer Cells from Human Pluripotent Stem Cells

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Human natural killer (NK) cells are an attractive source of lymphocytes for adoptive immunotherapy and can achieve durable remissions in patients with poor-prognosis acute myelogenous leukemia (AML). In order to generate NK cells that are effective against a broader range of malignancies, our lab has focused on the generation of NK cells from human pluripotent stem cells. We have previously demonstrated the potency of NK cells derived from human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) both in vitro and in vivo. These previous studies utilized a stromal cell co-culture system to derive hematopoietic progenitor cells ($CD34^{+}CD45^{+}$ cells) that can then produce $CD45^{+}CD56^{+}$ NK cells in a secondary culture system. Now, we have used a completely defined, spin embryoid body (spin-EB) approach potentially suitable for clinical scale-up. In these spin-EB cultures, defined numbers of undifferentiated hESCs or iPSCs are first aggregated in 96 well plates (3000 cells per well) in serum-free media containing the cytokines SCF, BMP4, and VEGF. Under these Stage 1 conditions, high frequencies of hematopoietic progenitor cells expressing CD34 (55.9 ± 6.4%), CD45 (26.2 ± 6.6%), and CD43 (41.8 ± 9.51%) develop and expand over 6-11 days. After this time, EBs are directly transferred (without dissociation or sorting) to Stage 2 cultures with EL08 stromal cells or stroma-free conditions in media containing NK cell-initiating cytokines. We find that these Stage 2 cells acquire all the typical markers of mature NK cells (CD56, CD94, KIRs and other surface receptors), secrete cytokines (IFNγ, TNFα), and kill leukemia, ovarian cancer, pancreatic cancer and multiple myeloma (MM) cell lines, as well as primary myeloma cells. Specifically, hESC (46.3%) and iPSC (42.4%) derived NK cells demonstrated significant anti-MM activity, comparable to peripheral blood NK cells. Notably, spin-EB derived NK cells generated in feeder free conditions also exhibit a typical phenotype, proliferate and kill a broad range of tumor targets. Interestingly, we found that feeder free cultures produce their own feeder layer capable of driving NK cell development. We have characterized these feeder layers expressing endothelial markers (CD31) as well as human leukocyte antigen (HLA) -A, -B, -C, and -E, which are important in the education and licensing of human NK cells. The feeder cells also support the development of mature, cytotoxic NK cells from umbilical cord blood HSCs. Ongoing work is focused on expansion and specific targeting of these large numbers of NK cells against a wide array of targets using antigen specific receptors.

102 (Cancelled)

Single Molecule Imaging of GPCR Oligomerization in Living Cells

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G-protein-coupled-receptors (GPCRs) are the largest receptor family in humans, and the largest class of drug targets. There is evidence that GPCRs form homo- and hetero-oligomers, and that these oligomers may show alterations in the affinity and specificity of ligand binding, as well as the activation of downstream signaling pathways. Thus, GPCR oligomerization is an important area of research for drug design. However, the physiological extent and significance of oligomerization in the largest subfamily A remains uncertain. Examination of oligomerization using standard fluorescence co-localization techniques is limited in spatial resolution, while ensemble measures of resonance energy transfer do not indicate the fraction of receptors that are interacting at a given time, or the dynamics of the interactions. Single-molecule approaches are capable of providing direct information about the spatial and temporal organization of receptor complexes in living cells. Thus far, there are only two published single-molecule studies of GPCR oligomerization in living cells, both of which were limited by the use of indirect labeling with fluorescent ligands, which might have affected oligomerization, as well as obscured analysis of the stoichiometry of oligomeric complexes. These studies suggested transient dimerization of the subfamily A M1 muscarinic receptor and formyl peptide receptor, with only 30-40% of the receptors estimated to exist as dimers at any moment. The present study directly images the subfamily A dopamine D2 receptor at the single-molecule level by covalent labeling with a single dye on each protomer and imaging by total internal reflectance fluorescence

microscopy in living cells. We measured fluorescence intensities in absolute numbers of photons per pixel per second and observed a distribution of intensity levels consistent with populations of various oligomeric states. These results were compared with the metabotropic glutamate receptor 2 (mGluR2), a subfamily C GPCR which is known to exist as covalently bound constitutive dimers. While the distribution of intensities of mGluR2 did not show an effect of temperature, the proportion of D2 receptors at the lowest intensity level increased at lower temperature, consistent with a decrease in collision-dependent transient oligomerization.

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Recombination Capacity of the Interferon Regulatory Factor 6 Allele Varies by Tissue

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Background: IRF6 is a member of the Interferon Regulatory Factor family of transcription factors. Mutations in *IRF6* cause Van der Woude (VWS) and Popliteal Pterygium Syndromes (PPS). A variant in *IRF6* also confers significant risk in developing Isolated cleft lip and palate. Mice that are homozygous for the null allele of *Irf6* (*Irf6*$^{gt/gt}$) demonstrate craniofacial, skin and limb defects. Also, mutations in IRF6 have been linked to squamous cell carcinoma. Since Irf6 functions in multiple tissues, we developed a conditional allele for tissue-specific excision in mouse (*Irf6*$^{fl/fl}$). We inserted LoxP sites in introns 2 and 4. Recombination between the *LoxP* sites is predicted to create a null allele because it deletes part of the DNA binding domain and creates a frameshift. Initially, we used the *EIIa-Cre* "deleter strain" to drive recombination of the floxed allele. The "Early II a" adenoviral promoter is used to drive *Cre* expression prior to implantation of the blastocyst in the uterine wall. However, we observed only 2/14 experimental embryos (*Irf6*$^{gt/fl}$*; EIIa-Cre*$^{+/-}$) had the knockout phenotype. To measure efficiency of recombination, we genotyped for the unrecombined floxed allele and the recombined null allele using genomic DNA. To date we have genotyped 10 of the 14 experimental embryos. We observed complete recombination in the two embryos with the knockout phenotype, but <100% recombination in the other 8 embryos as evidenced by persistence of the unrecombined floxed allele. To test whether recombination efficiency at the *Irf6* locus was tissue specific, we used a transgenic line with Cre expression under the Mouse Growth Differentiation Factor 9 (*Gdf9*) promoter. We choose this line because *Gdf9* is expressed in oocytes of primordial follicles by postnatal day 3. Thus, recombination would be available to occur when methylation of the genome is minimal and for an extended length of time (throughout oocyte differentiation and up to fertilization). Using the *Gdf9-Cre* line, we observed the knockout phenotype in 100% of experimental embryos (*Irf6*$^{gt/fl}$*; Gdf9-Cre*$^{+/-}$) (N=6). Our findings suggest that recombination of the *LoxP* sites at the *Irf6* locus was inhibited. While the mechanism of this inhibition is not known at this time, we note that recent studies show that Cre recombination can be inhibited by DNA methylation, and that expression of *Irf6* is regulated in part by DNA methylation. Further, our findings suggest that *Gdf9-Cre* transgenic line may provide a more valid test of recombination capacity of *LoxP* sites than the EIIa-Cre "deleter strain".

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A Novel Mouse Model of Retinitis Pigmentosa and Cone-Rod Dystrophy Associated with *Aipl1* Hinge Mutation

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Purpose: Defects in *Aipl1* (aryl hydrocarbon receptor interacting protein like-1) are associated with autosomal recessive Leber congenital amaurosis (LCA), the most severe and rapid of inherited retinal degenerations. The most common *Aipl1* defect is the nonsense W278X mutation, mimicked in the *Aipl1* null mouse. Interestingly, *Aipl1* defects are also linked to autosomal dominant juvenile retinitis pigmentosa and cone-rod dystrophy. One such mutation is P351Δ12, a 12-bp deletion in the primate specific C-terminal hinge region. The main goal of this work is to understand the mechanism behind *Aipl1* defects leading to dominant disease and the importance of the hinge region in function and survival of photoreceptor cells. **Methods:** Transgenic mice were generated expressing either N-terminal Flag tagged wildtype human AIPL1 (hAIPL1) or P351Δ12 hAIPL1, under the control of a 2.3 kb mouse *Crx* promoter, active beginning at E12.5 in both rod and cone photoreceptors (Furukawa et al., 2002). Transgenic positive founders were backcrossed with *Aipl1* null mice. Transgene expression was examined by immunoblotting with Flag and hAIPL1 antibodies. Photoreceptor function and survival was assessed by electroretinography (ERG) and immunocytochemistry. **Results:** Both mutant P351Δ12 hAIPL1 and wildtype hAIPL1 expressed similar protein levels. However, ERG responses of P351Δ12 hAIPL1 in an *Aipl1-/-* background showed an early dramatic reduction in cone and rod responses. In contrast, wildtype hAIPL1 fully rescued rod and cone vision in *Aipl1-/-* mice into late adulthood. Further biochemical and morphological analyses are being conducted to examine the cause of visual dysfunction associated with mutant P351Δ12 hAIPL1. **Conclusions:** This novel model of retinal degeneration associated with P351Δ12 AIPL1 mutation shows visual deficits and degeneration that is less drastic than *Aipl1* null mice. This indicates the greater treatment potential of gene therapy for patients with mutations in this region as compared to the common nonsense W278X AIPL1 mutation. Mutant mice showed a greater detriment to cone photoreceptors, in agreement with cone-rod dystrophy associated with this mutation. The deficits observed in P351Δ12 hAIPL1 mice demonstrate the importance of the primate specific region in proper functioning of AIPL1 in photoreceptor cells.

Poster Abstracts

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BRAFV600E Mediates Papillary Thyroid Carcinoma Invasion through MMP-1 Induction

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The BRAFV600E mutation is found in approximately 60% of sporadic papillary thyroid carcinomas (PTC) and is associated with aggressive disease and poor prognosis. Here we investigated the role of BRAFV600E signaling in metalloproteinase (MMP) induction and tumor invasion in both clinical PTC samples and experimental cell culture models. Sixteen human PTC samples were stratified by the presence of the BRAFV600E mutation or wild type allele and analyzed by real-time qRT-PCR for MMP-1 and -3. The BRAFV600E mutation was associated with a statistically significant increase in MMP-1 expression (3.25 fold increase, $p<0.01$), but not MMP-3 (1.51 fold increase, $p>0.01$; Student's t-test). The chemical MEK1 inhibitor, U0126, and BRAF siRNA were used to interrupt the constitutively activated BRAFV600E signaling cascade and assess its effect on MMP expression and cell invasion. U0126 treatment inhibited BRAF signaling (decreased ERK phosphorylation) and significantly decreased MMP-1 expression in the BRAFV600E mutant cell lines, BCPAP (PTC), and 8505C (anaplastic thyroid carcinoma) as measured by gelatin zymography of conditioned media, and by western blot of cell lysate. Significantly, the BRAF wild type PTC cell lines, TPC-1, and Nthy-ori-1 did not express detectible MMP-1. Similar results were observed with the addition of BRAF siRNA to BCPAP cells. Functionality of BRAF inhibition was demonstrated by invasion/migration assays employing extracellular matrix coated chambers. Control BCPAP cells (DMSO vehicle control) demonstrated a percent invasion/migration of 64%±5, which was significantly reduced by U0126 (5%±3 $p=0.0002$), BRAF siRNA (25%±19 $p=0.0088$), and an inhibitory anti- MMP-1 antibody (4%±3 $p=0.002$), but not by a non-targeting siRNA (54%±13 $p=0.443$; results reported as invading cells/migrating cells x 100 ±SD, Student t-test); similar results obtained with 8505C. These data demonstrate a correlation between BRAFV600E mutant PTC and increased MMP-1 expression in clinical samples, and expose a functional and targetable relationship with an invasive phenotype, that may be exploited for clinical benefit.

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Akt Regulation of Skp2 in Adipogenesis

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Diabetes is the 6th leading cause of death in the US and is a growing epidemic around the world. Adipose, an insulin responsive tissue is a key player in mediating insulin sensitivity through the secretion of adipokines, such as leptin. Both *in vitro* cell culture models and *in vivo* animal models show that Akt plays an integral role in adipogenesis. For example, mouse embryonic fibroblasts (MEFs), a white adipose tissue model & pre brown adipocytes, a brown adipose tissue model (BAT), lacking Akt, are hindered in their ability to differentiate into adipocytes. Mice that lack Akt2 have impeded adipogenesis & diabetes. In humans, a family with a missense mutation in Akt2 exhibited severe insulin resistance & lipodystrophy. However, the exact mechanism by which Akt regulates adipogenesis is unclear. Here, we elucidate one downstream target, Skp2 that is important for adipogenesis. Skp2 regulates adipogenesis by promoting mitotic clonal expansion (MCE). MCE is characterized by one to two rounds of proliferation, which has been reported to be important for initiating the adipogenic gene transcription program. We found that Akt upregulated Skp2 during MCE via mRNA translation that was mTORC1 dependent. Akt 1/2 DKO cells fail to upregulate Skp2 mRNA translation, fail to undergo MCE and fail to differentiate. Restoration of Skp2 in Akt 1/2 DKO cells is sufficient to rescue MCE and differentiation in Akt 1/2 DKO cells. However, even though Skp2 is able to restore adipocyte differentiation of Akt1/Akt2 DKO preadipocytes, it is not sufficient to restore the full functionality of mature adipocytes, as evidenced by the failure to upregulate various adipokines, failure to rescue GLUT4 expression, as well as failure to promote translocation of ectopic GLUT4. Thus, in addition to its role in the initiation of adipocyte differentiation, Akt is required for the full functionality of mature adipocytes.

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Design, Synthesis, and Analysis of Conjugated Activators for Modulation of Alternative Splicing

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Recent genome wide analysis has estimated that >95% of human genes are alternatively spliced, allowing for the production of many protein isoforms from a single gene. Splicing is controlled by SR proteins, factors which bind to exonic splicing enhancers (ESEs) through RNA binding domains. These proteins then recruit the splicosome via protein-protein interactions mediated by their RS domains, domains rich in highly charged arginine-serine dipeptides. Defects in alternative splicing have been linked to several diseases, including Cystic Fibrosis, breast cancer, and Spinal Muscular Atrophy (SMA). SMA is a neurodegenerative disorder caused by the deletion or mutation of the survival of motor neuron 1 (*SMN1*) gene. A nearly identical copy of the gene, *SMN2*, encodes an identical protein but contains a C→T transition on the ESE of exon 7, disrupting SR protein binding and resulting in substantial skipping of exon 7.

Of the many strategies for developing therapies for SMA, significant research efforts have focused on redirecting the splicing pattern of *SMN2* to produce a full-length SMN protein normally generated by *SMN1*. Splicing modulators have ranged from small molecule activators such as sodium phenylbutyrate and valproic acid, to viral vectors encoding antisense oligonucleotides. In particular, synthetic peptide-oligonucleotide conjugates containing short chains of arginine-serine dipeptides to mimic the RS domain has been shown to effectively redirect splicing patterns. However, little work has been done to explore the potential flexibility of the design of this synthetic RS domain. Herein, we report our efforts toward the design and synthesis of several synthetic SR protein mimics and the experimental design to determine their ability to increase

inclusion of exon 7 in *SMN2*. The synthetic RS domains consist of three motifs – α-peptide, β-peptide, and peptide dendrimer – attached to antisense oligonucleotides targeted to *SMN2* exon 7. The synthesis and conjugation of these mimics is straightforward, allowing for easy derivatization. Our results indicate that while neither synthetic RS domains nor antisense oligonucleotides alone affect splicing patterns, conjugated molecules appear to play a beneficial role at high nanomolar concentrations. We are currently synthesizing and examining derivatives of our initial design in order to further determine what combination of characteristics – such as number and arrangement of charged residues – constitutes the most effective splicing modulator.

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Mitochondrial Mutations Affect Early Steps In Colon and Rectal Adenocarcinoma Development

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Somatic mutations in tumor suppressor genes and oncogenes are known to drive malignant transformation, yet cancer cells must further adapt to the unique and dynamic metabolic requirements of the tumor microenvironment. One such adaptation is the well-established shift from oxidative phosphorylation to glycolysis. While somatic mutations in TCA enzymes have been recently implicated in several tumor types, an alternative mechanism of blunting oxidative phosphorylation is via mutation of the mitochondrial genome, which encodes critical subunits of the electron transport chain. Mitochondrial DNA (mtDNA) mutations have indeed been reported in some tumors, however, their spectrum across multiple tumor types and their role in tumor pathogenesis remain incompletely understood. Here we present the most comprehensive and unbiased description of the mitochondrial cancer genome to date, using whole-genome sequencing data generated by The Cancer Genome Atlas project to compare normal and tumor tissue mtDNA sequences from the same individuals. With median mitochondrial sequence coverage >1,200x, tumor-specific mutations and their precise levels of heteroplasmy were quantified. From 179 subjects with colon adenocarcinomas (n=84), rectal adenocarcinomas (n=43), acute myeloid leukemias (n=25), glioblastomas (n=18), and ovarian serous cystadenocarcinomas (n=9), we identified 125 somatic mtDNA mutations that alter protein-coding sequences (found in 84 tumors, or 47%) and 20 synonymous changes. This distribution of non-synonymous to synonymous mutations differed significantly from that observed among inherited variants (i.e. mitochondrial polymorphisms; P<2.2E-16). Somatic missense mutations in tumors were distributed uniformly among the mitochondrial protein genes but 63% of somatic truncating mutations occurred in ND5, including a recurrent frameshift mutation found in 16 individuals. Somatic mtDNA mutations were more prevalent in colorectal and ovarian cancers than in glioblastoma or acute myeloid leukemia (~75% vs ~30%; P=1.55E-08) and were equally prevalent in early and late-stage tumors. Thus, in some tumor types, mtDNA mutations likely arise and provide selective metabolic advantages in the earliest stages of tumorigenesis, eventually becoming fixed in the tumor both in number and level of heteroplasmy. As mtDNA mutations are easily detected in tumors and bodily fluids, specific mitochondrial mutations in ND5 have potential as early biomarkers of tumorigenesis in colorectal adenocarcinoma.

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Effects of Atropine and Timolol on Rabbit Episcleral Venous Pressure during Head-Down Tilt

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In humans, cranial venous pressure increases when going from an upright to supine position due to the hydrostatic column effect, but usually there is only a small change in episcleral venous pressure (EVP) and IOP. This suggests a regulatory mechanism may be isolating EVP and IOP from the effect of gravity during posture change. Because the episcleral circulation is innervated by both parasympathetic and sympathetic nerves, the goal of the present study was to test the hypothesis that EVP during head-down tilt is under muscarinic cholinergic or beta adrenergic neural control. In anesthetized rabbits (n=19), we measured mean arterial pressure (MAP), IOP, and orbital venous pressure (OVP) by direct cannulation; carotid blood flow (BFcar) by transit time ultrasound, heart rate (HR) by a digital cardiotachometer, and EVP with a servo-null micropressure system. Measurements were made initially with the animals in the supine position, and then with the animals tilted in a head down position after which the animals were given either atropine (0.5 mg/kg, iv bolus, n=8) or timolol (5 mg/ml, 50 microliters topical, n=11) while remaining in the head down position. The data (mean +/- standard error) were analyzed by repeated measures ANOVA. In both groups, head-down tilt caused significant ($p<0.05$) increases in OVP (0.9 ± 0.1 to 3.1 ± 0.3 mmHg), EVP (10.4 ± 0.4 to 12.4 ± 0.5 mmHg) and IOP (13.8 ± 0.8 to 16.2 ± 0.8 mmHg) while MAP (72.3 ± 0.9 to 71.6 ± 1.0 mmHg), BFcar (30.5 ± 2.2 to 29.6 ± 1.8 ml/min) and HR (293 ± 6 to 290 ± 6 bpm) were not significantly different. Timolol cause significant decreases in BFcar (11% ± 3%), HR (11% ± 2%) and IOP (17% ± 5%) but did not alter MAP or EVP. Atropine did not alter any of the measured parameters. Since neither atropine nor timolol affected EVP during head-down tilt, we conclude that EVP during posture change is not regulated by cholinergic or beta adrenergic neural control.

(Supported by the NGR of the American Health Assistance Foundation and the van Heuven endowment.)

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Loss of Fbx4 Cooperates With B-Raf to Induce Melanoma

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Dysregulation of cell cycle factors, such as cyclin D1, is a critical step in tumorigenesis. Increased cyclin D1 expression in cancer can be attributed to gene amplification, transcriptional activation, and impaired proteolysis. SCF^{Fbx4} is a ubiquitin ligase that

mediates degradation of phosphorylated cyclin D1 following the G1-S phase transition. The Fbx4 component of SCF^{Fbx4} confers specificity for cyclin D1 but requires an additional co-factor, αB-crystallin, for this function. Structural analyses reveal that the Fbx4 substrate-binding domain lies in the C-terminal region. We have identified a single amino acid mutation in our screen of over seventy melanomas in the substrate-binding domain residue I377M. This residue falls within the substrate-binding domain. Indeed, I377M Fbx4 substrate affinity is substantially decreased relative to wild-type Fbx4. Furthermore, both NIH 3T3 cells expressing exogenous I377M Fbx4 and human melanoma cells expressing endogenous I377M Fbx4 fail to efficiently ubiquitylate cyclin D1 and exhibit longer cyclin D1 half-life compared to wild-type Fbx4 expressing cells. Functionally, I377M Fbx4 is unable to efficiently regulate cell proliferation. Importantly, the inactivating I377M mutation correlated with nuclear accumulation of cyclin D1 in all four human melanoma cell lines examined, whereas cyclin D1 was detected abundantly in the cytoplasm of wild-type Fbx4 melanoma cells. Nuclear accumulation of cyclin D1 has been shown to be a transforming event *in vitro* and *in vivo*. To address the functional contribution of Fbx4 to suppression of melanoma, we have intercrossed Fbx4-null mice to conditional Braf(V600E/+) melanoma-prone mice. Braf(V600E) mutation will be induced upon topical administration of 4-hydroxytamoxifen. Without a cooperating genetic insult, mice bearing heterozygous mutations develop benign hyperplasias (nevi). These nevi do not progress to metastatic melanoma in the absence of a second genetic alteration. Intriguingly, our data indicate that loss of one or two copies of Fbx4 leads to tumor formation in mice harboring the Braf(V600E) driving mutation in found in human cancers. These insights highlight the need to develop effective pharmacologic inhibitors to supplement existing agents targeted at these pathways.

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Adeno-Associated Virus (AAV)-Mediated Gene Therapy Strategies for Articular Joint Restoration

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Articular cartilage has limited repair capacity, often leading to osteoarthritis (OA) after injuries. Sustained intra-articular (IA) delivery of therapeutic agents that can promote cartilage regeneration is of high clinical importance. Localized, stable and controllable *in vivo* transgene-expression following IA injection of AAV has been shown in intact rat joints. However, since undamaged joint is unlikely to require treatment, the study of AAV-delivery in models of joint injury is crucial to optimize therapeutic strategies. Interleukin-1 (IL-1) is thought to be a critical mediator of cartilage catabolism and thus plays a central role in the development of OA. IL-1 Receptor Antagonist Protein (IRAP) is a small protein that competitively inhibits IL-1. Although this strategy is straightforward, the efficacy is limited by poor pharmacokinetics. Therefore, IRAP is a promising candidate therapeutic gene for AAV-mediated cartilage regeneration. This study tests the hypotheses that persistent, localized, and controllable AAV-mediated transgene expression is possible within injured joints, and that AAV-IRAP therapy will have chondroprotective effects. Longitudinal study was performed by injecting AAV-Luciferase, driven by either CMV or TRE promoter, into rat knees with surgically created joint injuries of high clinical relevance: osteochondral defect and anterior cruciate ligament transection. Also, two different AAV-injection timepoints were investigated: pre- and post-injury. Transgene-expression was persistent, localized, and controllable with oral doxycycline up to 6-months in all groups. However, when the joints were opened, the location of transgene-expression was different: knees with AAV injected post-injury had signal highly localized to the vicinity of the cartilage injury and repair tissue, whereas those with AAV injected pre-injury had more diffuse signal in IA soft-tissues. These findings were consistent between the two injury models. The differential transgene location between the two AAV-injection timepoints can be used to optimize AAV-mediated cartilage repair strategies. The highly localized expression following post-injury AAV-injection is advantageous for strategies that need to target repair cells. The global expression following pre-injury AAV-injection provides a model to study the prophylactic delivery of protective factors to individuals that may be at high risk for cartilage damage. *In vitro* studies with rat chondrocyte pellets showed that AAV-IRAP abolished IL-1-induced catabolic effects of *mmp-3* and *-13* upregulation and glycosaminoglycan release. Overall, these data support further study of the *in vivo* therapeutic potential of AAV for safe and localized delivery of bioactive substances, such as IRAP. This can aid in cartilage repair, which may delay or prevent the onset of debilitating OA.

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Whole Exome Sequencing of Hepatocellular Carcinoma and Acute Myeloid Leukemia Identified *ARID2* and *BCORL1* as Two Novel Tumor Suppressor Genes

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Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related death worldwide. Through exomic sequencing of ten hepatitis C virus (HCV)-associated hepatocellular carcinomas (HCC) and subsequent evaluation of additional 129 affected individuals, we discovered novel inactivating mutations of *ARID2* in four major subtypes of HCC (HCV-associated HCC, hepatitis B virus-associated HCC, alcohol-associated HCC and HCC with no known etiology). *ARID2* encodes a subunit of the polybromo- and BRG1-associated factor (PBAF) chromatin remodeling complex. All *ARID2* mutations in HCC were inactivating mutations that were predicted to truncate the encoded protein. Notably, 18.2% of individuals with HCV-associated HCC in the United States and Europe harbored *ARID2* mutations, suggesting that *ARID2* is a tumor suppressor gene that is commonly mutated in this tumor subtype. To further our understanding of the genetic basis of acute myelogenous leukemia (AML), we determined the exomic

sequences of eight secondary AML specimens. We identified novel recurrent somatic mutations in the transcriptional corepressor gene *BCORL1* located on the X-chromosome. Analysis of *BCORL1* in an unselected cohort of 173 AML patients identified a total of 10 mutated cases (6%) with *BCORL1* mutations, whereas analysis of 19 AML cell lines uncovered 4 (21%) *BCORL1* mutated cell lines. The majorities (87%) of the mutations in *BCORL1* were inactivation mutations that were predicted to truncate the encoded protein. These results suggest that *BCORL1* by genetic criteria is a novel tumor suppressor gene.

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The Roles of MicroRNAs in Schwann Cell Differentiation

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Schwann cells (SCs) play important roles in the peripheral nervous system. As demonstrated in many demyelinating neuropathies, proper differentiation of SCs is essential for producing myelin sheaths around axons to increase the speed of axon conduction and allow proper function of the nervous system. Moreover, the plasticity of SCs allows SCs to dedifferentiate back to the immature promyelinating state upon nerve injury so that neuronal survival and axonal regeneration can be maximized. Since there are many human diseases and conditions, such as congenital hypomyelination, Charcot-Marie-Tooth disease and nerve trauma, that can be ameliorated through enhancing SC functions, it is imperative to understand the molecular mechanisms underlying SC differentiation. SC differentiation and dedifferentiation are thought to be "mirror images" of each other, in which the same set of positive and negative regulators of myelination are being actively regulated in both processes. Recently, it has been found that microRNAs (miRNAs) are also crucial in the regulation of SC differentiation during development. Mice with SCs lacking Dicer, which is believed to be required for processing most of the miRNAs, resemble the phenotype of Egr2-deficient mice, both of which display a severe neurological impairment mimicking congenital hypomyelination. We aim to identify key miRNAs that may be responsible for this phenotype by selecting miRNAs that are 1) developmentally upregulated in SCs, 2) downregulated in mice lacking Dicer, 3) predicted bioinformatically to target negative regulators of myelination, including Sox2, Jun and Notch1, 4) downregulated in injured nerves, and 5) induced by the master regulator of myelination, Egr2. We hypothesize that Egr2 represses antecedent gene programs, at least in part, through miRNA-mediated repression. The loss of the key miRNAs will render Sox2, Jun and Notch1 unrepressed and thus, prohibit SCs from progressing through the promyelinating-myelinating transition.

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Micrornas Regulate Drug Resistance and Spontaneous Metastases in Orthotopic Xenograft Models

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MicroRNAs (miRNAs) have emerged as important regulators of cancer initiation and progression.To examine the role of miRNAs in breast cancer progression, we profiled miRNA and gene expression in both clinical breast tumors and human-in-mouse breast tumor models, where breast cancer stem cells (BCSCs) contribute to spontaneous metastasis. We identified a limited number of miRNAs that are differentially expressed in metastatic triple-negative breast tumors and regulate drug resistance and tumor invasion *in vitro*. To facilitate miRNA functional studies in vivo, we also developed tumor imaging approaches by transducing BCSCs with optical reporter fusion genes (Luc2-eGFP or –tdTomato), which enabled both bioluminescence imaging (BLI) and FACS-based analysis and sorting. With non-invasive BLI approaches, as few as 10 cells of stably labeled BCSCs can be tracked *in vivo*. Using this model system and imaging technology, we have screened and identified miRNAs that regulate BCSCs, metastasis and drug resistance by targeting polycomb repressors (*BMI1*) and cytoskeleton genes (*TWF1* and *VIM*). Clinical studies demonstrated that the expression of candidate miRNAs was associated with and their target genes, indicating the clinical importance of the miRNA-gene network in breast cancer.

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NAD+ Bioenergy Sirtuin Sensors Integrate Metabolic and Inflammatory Switches in Human and Murine Sepsis

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Sepsis is a lethal acute inflammatory disease with distinct early hyper- and late hypo-inflammatory stages. No effective therapies beyond antibiotic administration and cardiopulmonary physiologic support exist. Concomitant with its clinical phase switch, we previously reported deactivated human and murine septic leukocytes shift from RelA/p65-dependent pro- to RelB-dependent anti-inflammatory responses. Energy sensor SirT1 integrates a bioenergetic transition and epigenetic gene-selective reprogramming to control the inflammatory switch. Here, we hypothesized bioenergy sirtuin sensors also reprogram metabolism during the sepsis response and discovered that SirT1 and SirT6 coordinate a switch from early glucose to later fatty acid oxidation as energy sources in human and murine septic leukocytes. Using THP1 promonocytic cell model of sepsis, we defined the mechanisms responsible for this metabolic flexibility. TLR4 activation rapidly reduces mitochondrial glucose oxidation by deactivatng pyruvate dehydrogenase (PDH) by phosphorylating pyruvate dehydrogenase kinase (PDHK). TLR4 signaling

simultaneously stimulates Warburg-like glycolytic metabolism by induction of glucose transporter, 6-phosphofructose kinases and lactate dehydrogenase. While the pro-inflammatory response switches to anti-inflammatory phase, glucose metabolism shifts to fatty acid oxidation by increases in synthesis of CD36 fatty acid transporter and carnitine palmitoyl transporter 1 (CPT-1). Hypoxia inducing factor-1α (HIF1α) and PPAR γ co-activators (PGC1α and β) control expression of glucose and fatty acid pathways, respectively. The metabolic switch was NAD+ dependent; wherein, SirT6 predominantly controlled glucose and SirT1 principally regulated fatty acid metabolism. Inhibition of glucose metabolism, but not the fatty acid metabolism, limits the scale of the inflammatory responses. We conclude that integration of epigenetics, bioenergetics and metabolism directs sepsis. Appropriate manipulation of metabolism, especially in the late anti-inflammatory stage, may improve clinical outcomes of this highly lethal disease.

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Novel Lipid-independent Serum Biomarkers for Genetic Risk of Coronary Atherosclerotic Disease

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Coronary atherosclerotic disease (CAD) is the leading cause of human mortality worldwide. While LDL is a major factor in pathogenesis of atherosclerosis, accumulative evidences suggest other heritable factors may play important role in this process. Recently, multiple unbiased genome-wide association studies have linked single nucleotide polymorphisms on chromosome 9p21 to atherosclerotic disease. The risk haplotype confers a 2 fold increase of myocardial infarction and is not associated with "classical" risk factors such as lipid levels, hypertension, obesity and tobacco use. Our subsequent work showed that reduced expression of *INK4/ARF* locus in lymphocytes is associated with the 9p21 risk allele. Another recent study found that an enhancer interval containing the 9p21 CAD risk locus physically interacts with the *INK4/ARF* locus and an interval downstream of type I IFN gene cluster. Here we report the identification of type I IFN subtypes as the serum biomarkers of 9p21 CAD risk. By examining protein and mRNA expression of type I IFNs in 170 random collected individuals, we found that the protein expression of both IFNα21 and IFNω was associated with rs10757278 CAD risk allele independently from age, gender and other co-morbidities such as diabetes. Moreover, mRNA expression of these IFNs also independently correlated with the expression of *INK4/ARF* and its regulator circular non-coding RNA (cANRIL) indicating possible synergy of cell cycle regulation and inflammatory pathway in athrogenesis. In addition, serum IFNα21 level was elevated in a small subset of individuals with CAD compared to age-matched healthy controls. These data indicate that IFNα21 and IFNω may act as novel lipid-independent serum biomarkers for CAD risk prediction and management. Future large-scale prospective study aiming to characterize the relationship of these biomarkers with CAD onset, severity and prognosis is essential to establish a more comprehensive CAD risk management strategy by integrating both serum cholesterol and these novel lipid-independent serum biomarkers of genetic CAD risk.

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Validation of Using Fingerstick Blood Sample with I-STAT POCT Device for Cardiac Troponin I Assay

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Background: In patients experiencing acute coronary syndrome (ACS), prompt diagnosis is critical in achieving the best health outcome. While ECG analysis is usually sufficient to diagnose ACS in cases of ST elevation, ACS without ST elevation is reliably diagnosed through serial testing of cardiac troponin I (cTnI). Point-of-care testing (POCT) for cTnI by venipuncture has been proven a more rapid means to diagnosis than central laboratory testing. Implementing fingerstick testing for cTnI in place of standard venipuncture methods would allow for faster and easier procurement of patients' cTnI levels, as well as increase the likelihood of starting a rapid test for cTnI in the pre-hospital setting, which could allow for even earlier diagnosis of ACS. **Objectives:** To determine if fingerstick blood samples yield accurate and reliable troponin measurements compared to conventional venous blood draws using the i-STAT POC device. **Methods:** This experimental study was performed in the ED of a quaternary care suburban medical center between June-August 2011. Fingerstick blood samples were obtained from adult ED patients for whom standard (venipuncture) POC troponin testing was ordered. The time between fingerstick and standard draws was kept as narrow as possible. cTnI assays were performed at the bedside using the i-STAT 1 (Abbott Point of Care). **Results:** 94 samples from 87 patients were analyzed by both fingerstick and standard ED POCT methods (see Table 1). Four resulted in cartridge error. Compared to "gold standard" ED POCT, fingerstick testing has a positive predictive value of 100%, negative predictive value of 96%, sensitivity of 79%, and specificity of 100%. No significant difference in cTnI level was found between the two methods, with a nonparametric intraclass correlation coefficient of 0.994 (95% CI 0.992-0.996, p-value < 0.001). **Conclusions:** Whole blood fingerstick cTnI testing using the i-STAT device is suitable for rapid evaluation of cTnI level in pre-hospital and ED settings. However, results must be interpreted with caution if they are within a narrow territory of the cutoff for normal vs. elevated levels. Additional testing on a larger sample would be beneficial. The practicality and clinical benefit of using fingerstick cTnI testing in the EMS setting must still be assessed.

Poster Abstracts

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The Tibetan PHD2 Asp4Glu is Associated with Hypersensitivity of Erythroid Progenitors to EPO and Upregulation of HIF-1 Regulated Genes Hexokinase (HK1) and Glucose Transporter 1 (SLC2A/GLUT1)

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The hypoxic response, mediated by hypoxia inducible transcription factors (HIFs), is central to the control and development of many essential biological functions, including erythropoiesis. As a high altitude population, many Tibetans, have developed a remarkable ability to protect against several hypoxic complications, including polycythemia as well as other harmful responses exhibited by non-adapted populations upon exposure to severe hypoxia. We have identified 10 genes involved in high-altitude adaptation in Tibetans, including a principal negative regulator of HIF-1α and HIF-2α peptides, i.e. PHD2 (EGLN1), as well as HIF2A (EPAS1) (Simonson, Science 2010). We reported a novel PHD2 Asp4Glu mutation that we found in 57 of 94 Tibetan (Lorenzo, Abstract# 2602 ASH 2010), 2 of 88 Asian and 0 of 38 Caucasian chromosomes. In most Tibetan samples, this variant is associated with a previously reported, unvalidated PHD2 polymorphism, Cys127Ser (found in 70 of 94 Tibetan, 27 of 88 Asian and 4 of 38 Caucasian chromosomes). To study the functional consequences of this PHD2 Asp4Glu mutation, we recruited seven Tibetan volunteers living in Utah, six of whom were homozygous and 1 heterozygous for PHD2 Asp4Glu and Cys127Ser. We unexpectedly found that homozygotes for the exon 1 PHD2 mutation had markedly hypersensitive erythroid BFU-E when compared to the normal range of normal controls we have standardized over several decades. Interestingly, erythroid progenitors from individuals with Chuvash polycythemia or a HIF-2α gain-of-function mutation also have hypersensitive BFU-E. To determine if the Tibetan erythroid hypersensitivity data may be explained by increased HIF activity, we have quantified HIF target gene expression in subject granulocytes and found a significant increase in hexokinase (HK1) and glucose transporter (GLUT1/SLC2A) mRNA levels.
These data report the first molecular defect with functional consequences that is associated with the complex Tibetan adaptation to extreme hypoxia.

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Antigen Presenting Potential: Human Mast Cells Activate T Cells via HLA/TCR Interactions

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Recent findings have shown that mast cells are multifaceted cells with the ability to respond to a variety of environmental signals and the potential to regulate inflammation in both atopic and non-atopic diseases. Because their numbers are elevated at sites of inflammation, mast cell interactions with other immune cells may be central to both acute and chronic diseases. The aim of this study is to determine the mechanisms through which mast cells interact with T cells, starting with the ability of mast cells to activate T cells via human leukocyte antigen (HLA)- T cell receptor (TCR) interactions. **Methods:** purified skin mast cells are analyzed for HLA-DR and costimulatory (CD80, CD83) molecule expression by fluorescent staining at baseline and after 3 days interferon gamma (IFNg, 10 ng/mL) stimulation. Mast cells, IFNg primed or not, are placed in co-culture with Jurkat cells, a human T cell line, for 3 days with or without superantigens Toxic Shock Syndrome Toxin-1 (TSST-1, which does not bind the Jurkat TCR V_{beta} region) and Staphylococcus Enterotoxin E (SEE, which does bind the Jurkat TCR). Jurkat expression of CD69 early activation marker is assessed by fluorescent staining. Mast Cells are placed in coculture with donor-matched T cells purified from peripheral blood with or without TSST-1. T cell activation is assessed by fluorescent staining of CD69 and proliferation is assessed by CFSE. **Results:** Mast cells stimulated with IFNg increase expression of HLA DR and CD80, but not CD83. IFNg primed mast cells, but not un-primed mast cells, activate Jurkat cells in the context of SEE, but not TSST-1, in a dose dependent manner. IFNg primed mast cells activate and cause the proliferation of donor-matched primary T cells in the context of TSST-1. CONCLUSIONS: The results of this study shows that though skin mast cells do not express HLA-DR at baseline, they do increase its expression on stimulation with physiologically relevant levels of IFNg and that IFNg primed mast cells can activate both Jurkat cells and primary human T cells via HLA/TCR interactions. If HLA/TCR interactions are mediated by superantigen, they are not antigen specific. The expression of CD80, a costimulatory molecule required for antigen specific activation of T cells, indicates that mast cells have the potential to act as true antigen presenting cells, an idea that warrants further investigation.

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Caspase-9: A Candidate Susceptibility Factor in Murine Alkylator-Induced Acute Myeloid Leukemia

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Therapy related acute myeloid leukemia (tAML) is a severe form of AML resulting from exposure to chemotherapeutics, such as alkylators, used as treatment for other malignancies. tAML makes up 10-20% of new AML cases and the incidence is rising, but disease outcome remain poor. Only a small percentage of patients exposed to alkylators develop tAML, and little is known about what predisposes patients to tAML. Evidence suggests a genetic component to tAML risk, but genetic alterations predisposing to tAML are largely unknown. Our lab has used a murine model to study tAML and has found that susceptibility to alkylator-induced leukemia varies by strain. Expression profiling of c-kit+/lineage- (KL) hematopoietic stem and progenitor cells from twenty mouse strains revealed a correlation between strain-dependent expression differences and tAML susceptibility. The single most differentially expressed gene between susceptible and resistant strains was Caspase-9 (Casp9), the initiator caspase of the intrinsic apoptotic cascade. Casp9 expression, as measured by microarray and qRT-PCR, is significantly lower in tAML susceptible strains (DBA/2J and PL/J) compared with a resistant strain (C57Bl/6J). PCR amplification of Casp9 cDNA from DBA/2J and PL/J also revealed expression of two alternatively spliced

variants predicted to encode truncated proteins. We predict that lower Casp9 expression in these strains would render cells more resistant to alkylator-induced apoptosis and, thus, more likely to acquire mutations leading to leukemogenesis. Flow cytometric apoptosis assays have shown that KL cells from DBA/2J mice are more resistant to apoptosis than KL cells from C57Bl/6J mice, following treatment with the alkylator N-ethyl-N-nitrosurea (ENU). To determine the functionality of the variant DBA/2J isoforms, each cDNA was expressed independently in Casp9 null fetal liver cells, which are resistant to ENU-induced apoptosis, and the apoptotic response of these transfected cells to ENU was analyzed. Expression of C57Bl/6J Casp9 cDNA restores wild type levels of apoptosis in Casp9 null cells. However, none of the isoforms of DBA/2J Casp9 can restore apoptosis in these cells. This suggests that reduced expression of Casp9 and decreased apoptosis in susceptible strains are due to genetic and/or splicing variants in the Casp9 gene. We hypothesize that such variants are ultimately responsible for the differential risk of tAML seen in the DBA/2J strain. Ultimately, defects in apoptosis may be relevant for human tAML susceptibility.

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Improving the Accuracy of SPECT Left Ventricular Scar Burden Assessment

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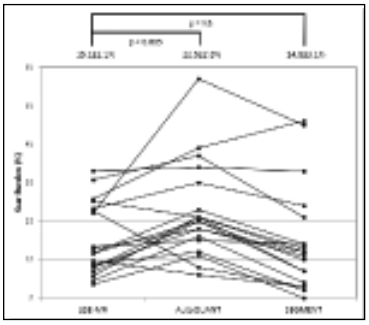
Background: Given its wide availability and technical simplicity, SPECT imaging has emerged as an important tool for decision making as to candidacy for interventional procedures, including revascularization and cardiac resynchronization therapy, in which left ventricular (LV) scar burden is a key consideration. In reviewing a patient who underwent both SPECT and late gadolinium enhancement cardiovascular magnetic resonance (LGE-MR) imaging, we observed that our commercial SPECT software (AutoQUANT, Philips) overestimated burden to a significant degree; further evaluation revealed this to be a systemic issue. To address this, we adapted an open-source software suite (SEGMENT [http://*segment.heiberg.se*]) to more accurately quantify scar burden. **Methods:** 19 patients with post-myocardial infarction scar underwent SPECT and contemporaneous LGE-MR. Using MR images, LV scar burden was quantified using QMass (Medis). In each patient, scar burden was quantified automatically using both AutoQUANT (which identifies scar based on extent [size of defect below threshold] only) and SEGMENT (which we adapted to identify scar based on both extent and severity of tracer uptake diminishment). **Results (Figure):** Using LGE-MR as a gold standard, SEGMENT was significantly more accurate than AutoQUANT in portraying LV scar burden. **Conclusions:** SPECT assay of scar burden using current commercial software is prone to overestimation. Consideration of perfusion defect extent and severity improves accuracy.



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A Novel Mouse Mutant for Cystic Kidney Disease and Defects of Planar Cell Polarity

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Background: Cystic kidney diseases represent the primary genetic cause of end-stage renal failure in North America, contributing up to 5% of incident cases. The molecular basis of cyst development is complex; recently, defects in planar cell polarity (PCP) have been implicated. Here, we describe a new mouse model of cystic kidney disease with evidence for PCP involvement. **Method:** In an autosomal dominant ENU mutagenesis screen, we identified a heritable mouse mutation that causes renal cysts. **Results:** Using whole-genome SNP analysis, we isolated a small 3-MB critical region within mouse chromosome 6 containing 25 genes, none of which have been associated with cystic disease. We identified a single point variant in one of these genes by whole-exome next-generation resequencing. The protein encoded by this candidate gene is highly expressed in the renal tubules and localizes to the basal bodies and the primary cilia; dysfunction of these organelles is involved in the generation of renal cysts. Histologically, heterozygous mutants demonstrate variable and often striking glomerular cysts. At a lower frequency, cysts are also identified along the entire nephron. Homozygous mutants die perinatally in the first few hours of birth. They exhibit renal cysts much earlier at the late embryonic stage. In addition, mutants have "kinked" tails and disoriented inner ear hair cells, phenotypes characteristic of PCP defects. Canonical Wnt signaling pathway, a known mediator of renal cysts, does not appear to play a role in the mutant phenotype as no appreciable differences are observed in the expression of β-catenin or its downstream effectors, Lef and Tcf, between mutants and wild type littermates. **Summary:** Taken together, we report a novel mouse mutant for autosomal dominant renal cystic disease, allowing further elucidation of the role of PCP and primary cilia in cystic kidney diseases.

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Development of a Stable Isotope Lipidomic Approach to HDL-Mediated Cholesterol Efflux

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The reverse cholesterol transport (RCT) pathway has proven to be a critical route by which unesterified (free) cholesterol is removed from macrophages, where its buildup can be toxic to the cells. HDL is a key mediator of RCT from macrophages to the liver, where cholesterol is processed and excreted in the bile. However, the current methods for following cholesterol efflux in cell cultures rely on radiolabeled cholesterol and cannot differentiate cholesteryl ester (CE) species produced intracellularly and extracellularly. To

improve detection specificity over existing [^{3}H] cholesterol assays, as well as, provide a method for eventually following cholesterol efflux *in vivo*, we sought to create a stable isotope efflux assay using nonradioactive deuterated [d_7]-cholesterol. This assay would be able to detect total labeled cholesterol as well as specific deuterated CEs by a lipidomic approach: electrospray ionization tandem mass spectrometry of acetyl chloride-derivatized cholesterol (e.g., acetyl CE) as well as endogenous CE molecular species. J774 macrophages were labeled with [d_7]-cholesterol, and efflux was measured to media containing different acceptors over a 6hr efflux time. Approximately 23% of total labeled cholesterol was found in media containing human plasma with BSA, ~12% in media containing HDL, and ~2% in BSA. Longer chain length CEs were also found by detection specifically through the neutral loss of a cholestane fragment (*m/z* 368.5), indicating that free cholesterol in plasma was being esterified by lecithin-cholesterol acyltransferase (LCAT). It was also found, however, that equimolar concentrations of varying chain length CEs exhibit differing MS responses; in general, the more unsaturated the chain, the higher the response signal. Further efflux studies utilizing a newly synthesized [d_7]-CE internal standard (i.e., 17:0-[d_7]-CE) and correcting for the response factors can enable quantitation and affinity of acceptors to individual CEs. This new assay for cholesterol efflux likely will be crucial to improve our understanding of cholesterol metabolism and the role of HDL in RCT.

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Low Serum Vitamin D is Associated with Metabolic Syndrome in African American and Caucasian American Male Veterans

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Introduction: Vitamin D and metabolic syndrome interaction has not been well studied; yet both define risk for developing cardiovascular disease (CVD). We assessed whether serum 25-hydroxyvitamin D (s25D) is associated with prevalent metabolic syndrome (MetS) and MetS components in a group of predominantly African American male (AAM) veterans at an urban veteran administration medical center (VAMC). **Methods:** Male veterans were recruited, s25D was measured, and health surveys and medical chart reviews were completed. Vitamin D deficiency and insufficiency were defined as s25D < 20 and < 30 ng/ml, respectively. **Results:** Among 923 men prevalence of MetS was 35% (n = 323). Race distribution was 65.5, 28.1, 5.5, and 0.9% for AAs, Caucasian Americans (CA), Hispanics, and other, respectively, reflecting diversity of our population. Majority of men were younger than 70 years (76%), retired (61%), considered themselves to be in good health (54.8%), and had low (< 30 ng/ml) s25D level (79%). Overall, 76% of males had a higher than normal BMI of 25 kg/m^2, with 35% being overweight and 41% being obese. We compared men without (MetS-) and with (MetS+) metabolic syndrome. Some of significantly different (p < 0.01) comparisons (MetS- vs MetS+) included current alcohol use (28.7 vs 15.2%), been married (32.4 vs 41.9%), retired (57.3 vs 67.3%), and health self-perception been fair/poor (40.7 vs 53.1%). Mean (standard deviation, SD) level of s25D in MetS- compared to MetS+ was 22.3 (9.3) and 18.5 (7.6) ng/mL, respectively (p < 0.001). The serum 25D decreased (p for trend <0.05), as the number of components of metabolic syndrome increased. Prevalence of metabolic syndrome in AA (36.1%) and CA (30.6%) was similar while s25D level was lower in AA (mean [SD] 18.8 [11.8] ng/ml) vs CA (25.7 [13.3] ng/ml, p<0.01) men. In multivariate regression analysis of the entire group, after controlling for other variables, independent determinants of metabolic syndrome included s25D level, current alcohol use, marital and working status, and self-perception of health. In the model, an increase in s25D of 1 ng/ml was associated with 4% decrease in odds of having metabolic syndrome. **Conclusion:** Low vitamin D is a significant predictor of metabolic syndrome in African American and Caucasian American male veterans. There is racial variability in vitamin D/metabolic syndrome interaction as well as important contribution of psychosocial risks to metabolic syndrome. Complexities of interracial interactions and plausible bi- or multi-directional vitamin D/metabolic syndrome relations suggest that further research engaging approach defined as systems biology is warranted.

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Impaired Myocardial Response to Glucagon-Like Peptide 1 in Humans with Type 2 Diabetes Mellitus

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Background: Effects of GLP-1 to drive myocardial glucose uptake and cardiac work have been reported in animal models but not directly tested in humans. **Methods:** We measured effects of GLP-1(7-36) (1.5 pmol/kg/min IV) on myocardial glucose uptake (MGU), oxygen consumption (MVO2), and blood flow (MBF) using dynamic positron emission tomography. Diabetic subjects underwent 4 wk treatment intensification using diet/exercise and insulin. Studies took place under fasting conditions following a 10 hour study infusion. **Results:** Ten lean and 7 T2D subjects were studied under GLP-1 stimulation, and 6 lean subjects served as saline controls. Lean and T2D subjects differed as expected (BMI 24.6±3.5 vs 36.8±2.8 kg/m^2, p=0.03; MAP 84±3 vs 104±7 mmHg, p=0.02; insulin 76.8±11.4 vs 169.8±36.0 pmol/L, p=0.03) but by design were matched on fasting fuel concentrations (glucose 5.0±0.3 vs 5.0±0.2 mmol/L, p=0.91; NEFA 0.15±0.07vs 0.20±0.06 mmol/L, p=0.18) . GLP-1 infusion increased plasma GLP-1(7-36) concentrations equally in both groups (lean from 6.2±1.6 to 85±9.6; obese from 5.0±0.9 to 81.1±10.7; p=0.86 comparing groups) GLP-1 had no effect on MBF. GLP-1 significantly increased MVO2 in lean subjects (Table). MGU was elevated 4-fold by GLP-1 in lean subjects. MGU in the GLP-1 treated T2D group was not different

	Lean-Sal (1)	Lean-GLP1 (2)	T2D-GLP1 (3)	1 vs 2	2 vs 3
MVO2 (mL O2/min)	88±3	108±6	93±7	*p*=0.03	p=NS
MGU (umol/100ml/min)	21±13	90±27	12±6	*p*=0.03	p=0.02

than the lean saline control group, and markedly lower than GLP-1 treated lean subjects despite matched glucose and NEFA levels and hyperinsulinemia. **Conclusions:** These data indicate that GLP-1 effects on myocardial glucose uptake and cardiac work are impaired in the setting of T2D.

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Phospho-Regulation of E-Cadherin Trafficking and Stability

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Multi-cellular organisms require cadherin/catenin adhesion for proper cellular differentiation as well as tissue integrity and architecture. Classical cadherins, such as the prototypical E-cadherin (E-cad), mediate calcium-dependent homophilic interactions between cells at adherens junctions. Adherens junctions are not static structures; epithelial cells must regulate the amount of E-cad at the cell surface according to their morphogenic or adhesive requirements. This necessitates a delicate balance of endocytosis and exocytosis. The cytoplasmic domain of E-cad binds to β-catenin (β-cat), allowing for efficient delivery to the cell surface. Phosphorylation of this domain increases the affinity for β-cat by ~800-fold *in vitro*. However, the regulation of E-cad phosphorylation and its relationship to trafficking remain poorly defined. We show that *in vivo* cadherin phosphorylation can be monitored by following mobility properties of a cadherin cytoplasmic domain fragment (Cad_{cyto}) on SDS-PAGE gels. The slower fragment is phosphorylated, and β-cat interacts exclusively with this slower form in co-IP analysis. Forced membrane targeting of Cad_{cyto} results in more efficient phosphorylation, indicating that the responsible kinase(s) are localized at or near membranes. Three serines are required for generating the slower migrating form of Cad_{cyto}, for ^{32}[P]-incorporation, and for β-cat binding and stabilization. Additionally, a phosphorylation deficient E-cad mutant (3S>A) accumulates intracellularly in a post-ER compartment and does not reach the cell surface as efficiently as WT or a 3S>D phosphomimmic. Moreover, steady-state immunoblots show that 3S>A is not expressed as highly as WT and 3S>D. However, low protein levels of 3S>A are not due to lower mRNA levels, and protein stability studies demonstrate that 3S>A has a shorter half-life(~2 hrs) than WT or 3S>D(~5 hrs). Degradation of 3S>A is blocked by chloroquine, suggesting that phosphorylation of E-cad blocks its lysosomal degradation. Inhibitors of kinases previously implicated in cadherin phosphorylation (GSK3α/β, CK2, and CK1) fail to block the mobility shift, suggesting the involvement of other kinases. To identify the cadherin kinase, a dsRNA knock-down screen of all ~350 kinases in the *Drosophila* genome is underway, with the expectation that loss of a robust cadherin kinase will completely block the mobility shift of Cad_{cyto}. Identification of the cadherin kinase will facilitate studies into the regulatable nature of the β-cat/cadherin binding interaction and its role in cadherin trafficking.

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Effects on Ipsilateral Lower Limb Muscles Revealed With Stimulus Triggered Averaging Of EMG Activity in Macaque Monkeys

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Stimulus triggered averaging (StTA) of EMG activity is well established as an effective method for identifying both excitatory and inhibitory linkages between motor cortex cells and motoneurons (Cheney and Fetz, 1985). Using this approach, work from our laboratory has yielded a rich new knowledge of the synaptic organization between cortical cells and motoneurons of upper and lower limb muscles (Park et al, 2000; Hudson et al, 2010). Although output effects on muscle activity from contralateral primary motor cortex (M1) have been extensively documented using a variety of methods, studies of output effects from ipsilateral cortex have been much more limited. It is known that approximately 10% of the corticospinal axons descend ipsilaterally in the spinal cord (Dum and Strick, 1966). These ipsilaterally projecting axons have been shown to terminate in intermediate layers of the spinal cord and also directly in the motor nuclei of distal muscles suggesting a relatively direct pathway by which ipsilateral stimulation might produce effects on muscle activity. Ipsilateral deficits associated with hemiparetic stroke demonstrate the potential functional importance of the ipsilateral motor cortex (Yarosh et al., 2004). Despite the potential functional and clinical importance of the ipsilateral corticospinal projection, relatively little is known about the normal functional properties of this projection. The goal of this study was to use StTA of EMG activity to investigate the properties (sign, strength, latency and muscle distribution) of poststimulus effects on lower limb muscle activity elicited from ipsilateral M1 cortex. We compiled StTAs of EMG activity from 22 muscles of the hip, knee, ankle, and foot in rhesus monkeys (Macaca mulatta) during performance of a task which required the monkey to grip a manipulandum with its foot and produce leg extension-flexion movements against a light load. Compared to contralateral M1, mirror image sites in ipsilateral cortex produced effects that were broadly similar in sign and muscle distribution pattern. The minimum latencies of effects elicited from ipsilateral cortex were similar to those from contralateral cortex. The average magnitude of poststimulus facilitation from ipsilateral cortex was profoundly weaker than facilitation from contralateral cortex (9% vs 30%, peak increase over baseline at 120 μA). Although ipsilateral effects are much weaker, our latency data suggest that these effects are mediated, at least in part, through direct corticospinal connections.

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Dissociation of Biological Infectivity from Seeding Ability in Prions with Alternate Docking Mechanism

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Previous studies identified two mammalian prion protein (PrP) polybasic domains that bind the disease-associated conformer PrP^{Sc}, suggesting that these domains of cellular prion protein (PrP^{C}) serve as docking sites for PrP^{Sc} for prion propagation. To examine the role of polybasic domains in the context of full-length PrP^{C}, we used prion proteins lacking polybasic domains as substrates in serial protein misfolding cyclic amplification (sPMCA) reactions. After ~5 rounds of sPMCA, PrP^{Sc} molecules lacking the central polybasic domain (ΔC) were formed. In contrast to wild-type prions, ΔC-PrP^{Sc} prions bound to and induced conversion of all other polybasic domain mutant PrP^{C} into PrP^{Sc} molecules. Remarkably, ΔC-PrP^{Sc} and other polybasic domain-mutant PrP^{Sc} molecules were minimally infectious in bioassay, despite their ability to seed sPMCA reactions of normal mouse brain homogenate. Thus, ΔC-PrP^{Sc} prions interact with PrP^{C} molecules through a novel mechanism, yielding an expanded substrate range and highly efficient PrP^{Sc} propagation. Further, ΔC-PrP^{Sc} and other polybasic domain-deficient PrP^{Sc} molecules provide the first example of dissociation between brain homogenate sPMCA seeding ability and biological prion infectivity. These results suggest that the propagation of PrP^{Sc} molecules may not depend on a single stereotypic mechanism. Instead, multiple PrP^{C}/PrP^{Sc} interaction mechanisms exist, though a subset involving polybasic domains may be required to generate prion infectivity. Furthermore, recent findings suggest that prion formation involves stepwise PrP conformational change that includes polybasic domain rearrangement.

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Central Amydaloid Histone Deacetylase isoform 2 in Anxiety and Alcoholism

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Alcoholism is a complex psychiatric disorder with severe social and health consequences that is driven by an underlying genetic basis and comorbid psychiatric disorders such as anxiety. The alcohol-preferring (P) and-nonpreferring (NP) rat lines have been selectively-bred for higher and lower alcohol preference, respectively, and P rats display innate anxiety-like behaviors in comparison to NP rats, thus providing a well-established animal model for human alcoholism. Recently, epigenetic mechanisms, including histone deacetylase (HDAC)-mediated chromatin remodeling, have been implicated in changes in gene expression associated with synaptic plasticity, and it has been suggested that transgenerational epigenetic inheritance may contribute to behavioral phenotypes and psychopathologies. However the role of innate differences in chromatin structure in the genetic predisposition to anxiety and alcoholism has not yet been studied. We found that higher HDAC2 expression in the central nucleus of amygdala (CeA), a brain area implicated in both anxiety and alcoholism, produced deficits in histone acetylation and synaptic plasticity associated-gene expression and regulates anxiety and alcohol-drinking behaviors. These abnormal epigenetic marks in the amygdala of P rats were corrected by injection of acute ethanol, which also attenuated anxiety-like behaviors of P rats without an effect in NP rats. Furthermore, ChIP analysis of amygdaloid lysates found that ethanol injection increased acetylated H3-associated BDNF exon IV, but not exon I, and Arc genes in P rats, but not NP rats. We employed siRNA-mediated knockdown of HDAC2 in the CeA of P rats and found that the reduction of HDAC2 resulted in anxiolytic effects associated with increased H3 acetylation, expression of synaptically active genes, and dendritic spine density (DSD). HDAC2 siRNA infusion into CeA also reduced alcohol consumption in P rats. Our results demonstrate, for the first time, that innately higher expression of HDAC2 in the CeA results in condensed chromatin structure and regulates both anxiety and the anxiolytic effect of ethanol that plays a role in alcoholism. We anticipate that isoform specific HDAC2 inhibitors could serve as useful therapeutic agents for the treatment of comorbid anxiety and alcohol-use disorders.

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Targeted Nanodiamond-Liposome Hybrids Enhance Cancer Imaging and Therapy

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Note: Authors Moore and Chow contributed equally to this work.

Diamond nanoparticles (NDs) have been shown to be highly biocompatible vehicles for the delivery of therapeutic and imaging agents. By promoting internalization and retention of their cargo while limiting non-specific uptake during circulation NDs are able to increase the efficacy and reduce the toxicity of a number chemotherapeutic agents, such as doxorubicin and paclitaxel. In order to target cancer cells for imaging and therapy, we have developed a self-assembled nanodiamond-liposome hybrid particle (NDLP) that preserves the potent interactions between ND surfaces and drug or fluorophore. We report the synthesis, characterization and evaluation of epidermal growth factor receptor (EGFR) targeted NDLPs carrying either a fluorophore or epirubicin, for targeted imaging or therapy of breast cancer. Targeted delivery of NDs carrying a near-infrared fluorophore resulted in increased fluorescence retention in EGFR overexpressing breast cancer cells (MDA-MB-231) both *in vitro* and *in vivo*, as compared to cells not-overexpressing the receptor (MCF7). In cultured cells, NDLP-epirubicin effectively killed EGFR overexpressing cells while causing no change in the viability of cells not overexpressing the receptor. Most importantly, systemic delivery of anti-EGFR targeted NDLP-epirubicin prevented drug lethality and resulted in tumor regression in mouse breast cancer

xenografts (MDA-MB-231 cells). These targeted NDLPs represent a promising modality for imaging and treating a variety of cancers, including the triple negative breast cancers modeled here. Because the system is highly modular it can be readily modified to target nearly any cell surface receptors and carry a variety therapeutic agents, giving NDLPs the potential to improve the treatment of a vast number of human diseases.

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In Situ Labeling with 17-Carbon Sphingoid Bases is Regulated by Multiple Sphingolipid Enzymes

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Many enzymes use sphingoid bases as substrates. In mammals, these enzymes include ceramide synthases (CerS), sphingosine kinases (SK), and reverse ceramidase (CDase) activities. Many researchers have used radioactive or odd-chain sphingoid bases as *in situ* metabolic labels to study sphingolipid metabolism, yet the contribution of each of the sphingoid base-metabolizing enzymes to the acute uptake and incorporation of exogenous sphingosine (Sph) has not been well established. In this study, we investigated the uptake and incorporation of the odd-chain sphingoid base, 17-carbon Sph (17C-Sph) into ceramides using high performance liquid chromatography/mass spectrometry (LC/MS). Results show that after incubating cells with 17C-Sph for 30 minutes, C16:0-ceramide was the predominant ceramide species produced. Conversely, very long-chain ceramides were produced in low levels despite the high expression of CerS2 in MCF-7 cells and predominance of very long-chain CerS activity *in vitro*. The 17C16:0-CerS activity was further characterized as being sensitive to the CerS inhibitor fumonisin B1 and to knockdown of CerS5 and/or CerS6. We next investigated the role of reverse CDase activities (i.e., aCDase and nCDase) and discovered that, although neither aCDase or nCDase knockout fibroblasts had altered 17C16:0-ceramide formation, deficiency of either of these CDase reduced 17C-Sph-1-phosphate (17C-S1P) production. The effects of SK1 and SK2 deficiency on 17C-Sph metabolism were also examined, and the results showed that deficiency in either SK1 or SK2 reduced 17C-S1P formation; loss of each of these enzymes also affected ceramide generation in distinct ways. Overall, this study provides valuable insight into the metabolism of exogenous Sph and the use of Sph as an *in situ* metabolic label.

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The NIH Investment in Repurposing Clinical Data for Translational Medical Science

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Background: Despite faster than Moore's law progress in genomic sequencing technologies, high quality phenotypic and risk exposure data remain expensive and labor-intensive to acquire. Clinical research registries are well-established tools for obtaining such data, but the technical, policy, and funding approaches to their organization and operation have been largely ad hoc. We therefore undertook a systematic, cross-sectional review of active observational registry projects funded in the U.S. and provide a quantitative analysis of this landscape. **Methods:** We identified a comprehensive list of all actively U.S. funded research registry-related projects for FY2009, employing structured searches of data sources at NIH and other U.S. government websites (NIH RePORTER; Catalog of NIH Funded Databases, Disease Registries, and Biomedical Information Sources). Registry projects were classified and linked to fiscal expenditures (NIH from FY1992; CDC from FY1995) using publicly available data (http://USAspending.gov; https://fpds.gov; http://tags.hhs.gov). **Results:** We identified 161 'parent' projects active in FY2009, encompassing approximately 3300 individual grants and contracts from 1992-2009. During this 18-year period, total registry project-related expenditures were $3.16B (all figures consumer price index adjusted to U.S. 2008 dollars); FY2009-only funding was $374M. Overall duration of projects was 0 to 43 (mean 6.9) years. 42% of projects were registries only; 55% included a biorepository component. NIH institute-based, central data and biorepository projects received $256M (8% total). Project funding by institute or center (IC) ranged from $801M (NCI) to <$1M (NLM, Fogarty International Center), and relatively from 2.4% (NIA) to ≤0.01% (NEI, Fogarty) as percent of total IC funding. By NIH research, condition, and disease categorization (RCDC), spending on cancer projects was $1.52B and the greatest percentage-wise registry spending represented 19% of total funding for the American Indians/Alaska Natives RCDC. A limited analysis of publication output yielded a range of 0 to 18 publications per million dollars funded for the subset of NIH grants of >2 years duration. **Discussion:** Registries and biorepositories represent a significant investment in translational science. Given their growing importance as sources of high-quality phenotypic data, we propose the introduction of standardized metrics for evaluating registries, common information technology platforms for interoperable data collection, and policies facilitating broad reuse of meticulously collected data.

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Coupling of Cellular Barcoding with Next-Generation Sequencing Technology for High-Resolution Clonal Tracking and Characterization of Normal and Malignant Human Mammary Stem Cells

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Background: Recent evidence suggests a hierarchical model of differentiation in the normal human mammary gland, whereby self-renewing bipotent stem cells generate lineage-restricted progenitors and terminally differentiated cells of both myoepithelial and luminal lineages. However it is not yet clear whether remnants of this type of developmental structure persist in breast cancer cell populations although it has been reported that only a subset of phenotypically distinct cells have tumor-initiating ability.

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Methods/Results: To investigate potential differences in normal mammary stem cell properties (self-renewal and differentiation), and to track the tumor-forming activity of breast cancer stem cells in a quantitative clonal fashion, we are using a cellular barcoding strategy. The "barcodes" are 54 base pair sequences of non-coding DNA, cloned into a lentiviral vector to allow their singular incorporation into individual transduced cells. A large library of ~1 million bacterial clones was prepared, and shotgun sequencing of a first 230 of these showed each contains a unique barcode. Optimization of conditions for lentiviral transduction minimized the frequency of double barcode integrations, and demonstrated indistinguishable transduction efficiencies of luminal progenitor and basal mammary cells. Transplants into highly immunodeficient mice were used to assess the regenerative activity of barcoded basal (stem cell-enriched) populations, and the tumor-forming activity of barcoded metastatic breast cancer cells (obtained from a patient's metastatic pleural effusion that had already been propagated in an immunodeficient mouse). Barcoded progeny of these transplants have been processed and will be sequenced at a depth to detect clones of 20 cells using various controls to correct for inter-sample sequencing output fluctuations. **Conclusions:** We have produced a powerful tool for the simultaneous clonal tracking of large numbers of individual stem cells. The results of the present experiments will provide new insights into the range of proliferative activities exhibited by normal and malignant human mammary stem cells in an in vivo setting.
*LVN is supported by a Vanier Canada Graduate Scholarship.

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Identification of Micrornas Associated with Progeroid and Normal Aging in Mice

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MicroRNAs (miRNAs) are ~22 nt, single-stranded RNAs that dysregulate pathways by post-transcriptional regulation of genes. The role of miRNAs in cell senescence and aging is poorly understood. We are using Excision Repair Cross-Complementing 1 (ERCC1) deficient mice modeling the human Xeroderma Pigmentosum F-ERCC1 (XFE) progeroid syndrome to identify dysregulated miRNAs in aging. Individuals with XFE progeroid syndrome and Ercc1$^{-/-}$ mice exhibit rapid onset of degenerative changes seen in normal aging. We hypothesize that altered miRNA expression in Ercc1$^{-/-}$ primary mouse embryonic fibroblasts (MEFs) compared to wild-type (WT) MEFs contributes to senescence through changes to miRNA target genes. We used miRNA microarrays to obtain expression profiles of Ercc1$^{-/-}$ and WT MEFs grown in 3% or 20% O_2 conditions. We confirmed the array via individual quantitative RT-PCR (qRT-PCR) miRNA assays. Functional studies were performed on WT and Ercc1$^{-/-}$ MEFs that were transfected with either anti-miR-10b or pre-miR-10b to alter cellular expression of the miRNA. After RNA isolation, qRT-PCRs were performed measuring changes to microRNAs, IL-6, p16 and other genes induced by senescence. Microarray analysis showed 14 upregulated and 22 downregulated miRNAs in presenescent versus senescent Ercc1$^{-/-}$ MEFs grown in 3% O_2. These microarray data were validated using qRT-PCR quantification of miRNA expression. Six of the downregulated miRNAs (miR-455*, miR-497, miR-450B-3p, miR-10b, miR-128 and miR-449a) were also downregulated in liver tissues of both Ercc1$^{-/\Delta}$ and WT old mice relative to young WT mice. Functional studies are in progress to test whether knocking down these specific miRNAs in wild-type MEFs promotes cellular senescence by measuring changes to IL-6 and p16. We have shown that the miRNA expression profiles of Ercc1$^{-/-}$ MEFs versus WT MEFs are significantly different and a subset of these miRNAs are also differentially expressed in early versus late passage Ercc1$^{-/-}$ MEFs at 3% O_2. Six of these miRNAs are similarly dysregulated in WT and Ercc1$^{-/\Delta}$ mouse livers, emphasizing a possible role for them in aging. These miRNAs are promising targets to study how they are dysregulated in aging and alter known senescence pathways. **Funding Sources**: A pilot grant from University of Pittsburgh Cancer Institute and T32 AGAG21885

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MIDAS Motif Governs *Enterococcus Faecalis Endocarditis*- and *Biofilm-Associated Pilus* Function in Vivo

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Little is known about molecular mechanisms of virulence of the bacterial opportunist *Enterococcus faecalis*, a frequent cause of hospital-acquired infections (HAIs), including the most common HAI - urinary tract infection (UTI). Rising disease incidence and antibiotic resistance rates demand novel therapeutic targets, such as the heterotrimeric *endocarditis- and biofilm-associated pilus* (Ebp) - a member of the widespread gram-positive sortase-assembled pilus class. However, neither the structural nor functional basis of the Ebp's role in disease has been established. Here, we show that the Ebp and its putative tip pilin EbpA are involved in binding to human bladder epithelial cells *in vitro* and in pathogenesis in a mouse model of foreign body-associated UTI (fb-UTI). Further, the Ebp's function depends on EbpA's predicted metal ion-dependent adhesion site (MIDAS) – a metal-coordinating motif necessary for some integrins' binding to their extracellular matrix protein substrates. We created chromosomal deletion mutants of the structural pilins, EbpA, EbpB, and EbpC, and a triple point mutant in the MIDAS. T24 bladder cell monolayers were challenged with FITC-labeled bacteria for 2h. After washing, the proportion of cells with adherent bacteria was determined by flow cytometry. Female C57BL/6 mice were transurethrally infected with 10^7 CFU of bacteria after placement of a silicone intra-bladder implant to mimic a urinary catheter. 24h post-infection (pi), mice were sacrificed; implants and bladders were harvested for viable bacterial counting. A significantly lower proportion of T24 cells with adherent bacteria was observed from cells challenged with Δ*ebpA* and Δ*ebpABC* strains compared to the isogenic wild-type or Δ*ebpB* and Δ*ebpC* strains. Bladders and implants harvested from mice infected with single deletion mutants, Δ*ebpABC*, or the MIDAS mutant supported significantly fewer viable bacteria 24 h pi compared to wild-type. Our results show that *E. faecalis* adhesion to T24 cells *in vitro* depends on EbpA. Though each structural pilin is necessary for full virulence *in*

vivo, mutating only three residues of EbpA's MIDAS motif leads to a virulence defect as severe a pilus knock-out (Δ*ebpABC*), showing that EbpA's MIDAS motif is necessary for Ebp function in murine fb-UTI. These results are among the first suggestive of a mechanism for any gram-positive sortase-assembled pilus function in disease.

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Astrocyte-Elevated Gene-1 (AEG-1) is Induced by Hypoxia and Glucose Deprivation and Modulates the Glycolytic Phenotype in Glioblastoma

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Glioblastoma continues to rank among the most lethal primary human tumor. Despite treatment with the most rigorous surgical interventions along with the most optimal chemotherapeutic and radiation regimens, the median survival is just 12-15 months after diagnosis for patients with glioblastoma. One feature of glioblastoma associated with poor prognosis is the degree of hypoxia and expression levels of hypoxia-inducible factor-1 α (HIF-1α). HIF-1α expression allows metabolic adaptation to low oxygen availability, partly through upregulation of vascular endothelial growth factor (VEGF) and increased tumor angiogenesis. In this study, we demonstrate an induced level of astrocyte-elevated gene-1 (AEG-1) in high-grade as compared to low-grade astrocytomas and association of AEG-1 with necrotic areas in glioblastoma. AEG-1 was recently demonstrated to be an oncogene that can induce angiogenesis and HIF-1α expression in glioblastoma. Results from *in vitro* studies show that AEG-1 is induced by hypoxia in a HIF-1α-dependent manner and that PI3K inhibition abrogates AEG-1 induction during hypoxia. Furthermore, we show that AEG-1 is induced by glucose deprivation and that prevention of intracellular reactive oxygen species (ROS) accumulation prevents this induction. Additionally, AEG-1 knockdown results in increased, whereas AEG-1 overexpression results in decreased, ROS production and glucose deprivation-induced cytotoxicity, indicating that AEG-1 induction is necessary for cells to survive this type of cell stress. Moreover, AEG-1 modulates the expression of glycolytic enzymes in glioblastoma cells *in vitro* and *in vivo* and regulates the expression of these enzymes as well as glycolytic flux during metabolic stress, such as glucose deprivation. Studies in Nude mice demonstrate that AEG-1 knockdown reduces the growth of glioblastoma xenografts and also promotes chemosensitivity to glycolytic inhibition *in vitro*. These findings identify a novel role for AEG-1 in the regulation of glycolysis in glioblastoma and indicate that anti-glycolytic therapies may be useful in treating malignancies that demonstrate AEG-1-overexpression.

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Permanent Alterations in Recurrent Inhibition after Peripheral Nerve Injury

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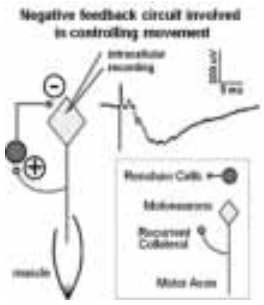
Peripheral nerve injury is frequently encountered by health professionals all over the world and serious functional disabilities persist despite successful peripheral regeneration. Interestingly, after successful regeneration, permanent central changes at the level of motoneuron synapses are observed. For example, in 1994, Cope TC et al. demonstrated that reinnervated muscles fail to produce stretch reflexes due in part poor recovery of neural circuit operation in the spinal cord. Further study indicates modification of multiple spinal circuits, including one which adjusts motoneuron activity through negative feedback. Motoneurons send their axons to innervate skeletal muscles in the periphery. In addition, they send intraspinal axon collaterals that synapse on inhibitory interneurons (Renshaw cells), the latter synapse on motoneurons resulting in their inhibition (see figure). This circuit is thought to limit and stabilize motoneuron firing rates. The injury of axons in the periphery prompted us to examine whether intraspinal collaterals are also permanently affected. We analyzed intraspinal axon collaterals of motoneurons that regenerated following nerve injury and surgical repair more than one year earlier. In all regenerated motoneurons there was a decrease in the number of collaterals and boutons per collateral suggesting permanent partial denervation of Renshaw cells coupled to medial gastrocnemius motor (MG) pools. We went on to show that these structural losses produced functional impairment. Intracellular records of recurrent inhibitory post synaptic potentials (RIPSPs) obtained from anesthetized rats *in vivo* demonstrated that in comparison with normal rats (n=23), ones with regenerate nerves exhibited major decreases (n=8) in RIPSPs mean amplitudes. In conclusion, permanent central changes in the recurrent inhibition circuit were detected despite successful peripheral nerve regeneration. The significant impairment of the negative feedback may explain the erratic and unusually high firing observed in motoneurons following nerve injury and repair. More generally, these findings demonstrate that regeneration fails to restore normal function to multiple spinal circuits, thereby indicating global, and rather permanent central adaptation/maladaptation of spinal cord circuitry in response to peripheral nerve injury.



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Radiation-Induced Changes in Gene Isoforms Identified by RNA-Sequencing

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Ionizing radiation (IR) is used increasingly in medical settings for diagnostic and treatment purposes, and environmental exposure is ubiquitous and unavoidable. An approach to improving the efficacy of radiation therapy and mitigating harmful effects involves modulation of molecular mediators of IR response. To achieve this goal, we need a comprehensive understanding of cellular response to radiation. To date, most analyses of IR response have focused on measuring gene expression changes using microarrays. Here, we used next generation RNA-sequencing to study the IR-induced changes in gene expression and splicing. We found changes in gene isoforms from alternate usage of promoters, splice sites, and 3' UTRs, in addition to induction and repression of gene expression levels. The nucleotide-resolution provided by sequencing allowed us to identify IR-responsive genes that were missed in previous studies. Specifically, we carried out RNA-sequencing of B-cells from 10 normal individuals, before and 2 and 6 hours following 10 Gy of IR exposure. We obtained 225 Gb of sequences, mapped them by TopHat to the human genome, and quantified expression of distinct gene isoforms using Cufflinks. We identified 1,062 genes whose expression levels changed following radiation exposure. In addition, we found 72 genes with IR-induced changes in gene isoforms; 39 of these genes exhibit changes in isoform without changes in overall gene expression levels. These include genes that play a role in cell senescence and DNA damage response including *RAD54B* and *RBBP8*. Also included are several genes with known roles in chromatin modification and remodeling such as *DNMT3A*, *ING3*, *PHF17*, and *SUV420H1*. These changes in gene isoforms were confirmed by western blots which showed different spliced forms of the encoded proteins before and following radiation exposure. Our results identify alternative splicing as a key component in cellular response to radiation. A comprehensive understanding of the transcriptional and processing steps engaged by human cells to deal with radiation exposure will improve the precision of radiation treatment and minimize damage to normal tissues.

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Genetically Encoded Fluorescent Sensors Reveal Changes in Zinc Homeostasis at Subcellular Resolution

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Zinc homeostasis is essential for normal human growth, development, and immunity. For example, zinc deficiency leads to stunting, diarrhea, cognitive changes, and immune dysfunction, and is responsible for 800,000 deaths every year of children under 5. However, it is poorly understood how perturbations in zinc homeostasis affect organisms at the cellular level and lead to clinical symptoms. Identification and characterization of zinc homeostasis regulators are necessary steps toward defining the role of zinc homeostasis in health and disease. We have engineered a family of ratiometric, genetically-encoded, fluorescent zinc sensors that allows us to observe changes in the exchangeable zinc concentration in different subcellular compartments of live cells. Our recent work includes the optimization of high dynamic range genetically encoded zinc sensors that are targeted to the mitochondrial matrix. These sensors have been tested in a variety of cell types, including HeLa cells, MIN6 cells, and primary hippocampal neurons, and they enable us to quantitatively compare the exchangeable zinc concentrations in mitochondria. We have begun to use our sensors to identify mitochondrial zinc transporters and to help us understand how mitochondrial zinc homeostasis affects cell signaling, growth, metabolism, and death. This work is supported by the National Institutes of Health (NIH) Grant GM084027 and NIH T32 GM008759.

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Kaposi's Sarcoma Associated Herpesvirus (KSHV) Latency Associated Nuclear Antigen (LANA-1) and Angiogenin Interact With Common Host Proteins Including Annexin A2 That Is Essential For The Survival Of Latently Infected Cells

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Kaposi's sarcoma-associated herpesvirus (KSHV) infection and latency-associated nuclear antigen (LANA-1) up-regulates the multifunctional protein angiogenin (ANG). Our studies demonstrate that silencing ANG or inhibiting its nuclear translocation down-regulates KSHV LANA-1 expression and ANG is necessary for KSHV latency, anti-apoptosis and angiogenesis (Sadagopan et al., J. Virol. 83:3342-3364, 2009; J Virol. 85:2666-2685. 2011). Here we show that LANA-1 interacts with ANG and colocalizes in latently infected endothelial cells, TIVE-LTC. Mass spectrometric analyses of TIVE-LTC proteins immunoprecipitated by anti-LANA-1 and ANG antibodies identified twenty-eight common cellular proteins such as ribosomal proteins, structural proteins, t-RNA synthetases, metabolic pathway enzymes, chaperons, transcription factors, anti-oxidants, and ubiquitin proteosome proteins. LANA-1 and ANG interaction with one of the proteins, annexin A2, was validated. A higher level of annexin A2 is expressed in KSHV (+), but not in EBV (+), B lymphoma cell lines. Annexin A2 colocalized with several LANA-1 punctate spots in KSHV (+) body-cavity B-cell lymphoma (BCBL-1) cells. In triple staining analyses, we observed annexin A2-ANG-LANA-1, annexin A2-ANG and ANG-LANA-1 colocalizations. Annexin A2 appeared as punctate nuclear dots in LANA-1 positive TIVE-LTC cells while in LANA-1 negative TIVE-LTC cells, it was detected predominately in the cytoplasm with some nuclear spots and colocalization with ANG was observed mostly in the cytoplasm. Annexin A2 co-immunoprecipitated with LANA-1 and ANG in TIVE-LTC and BCBL-1 cells as well as with ANG independent of LANA-1in 293T cells. This suggested that annexin A2 forms a complex with LANA-

1 and ANG as well as a separate complex with ANG. Silencing annexin A2 in BCBL-1 cells resulted in significant cell death, down-regulation of cell cycle associated Cdk6, cyclin D, E and A proteins, and down-regulation of LANA-1 and ANG expression. No effect was seen in KSHV (-) lymphoma (BJAB and Ramos) and 293T cells. These studies suggest that KSHV is probably using annexin A2 to maintain the viability and cell cycle regulation of latently infected cells. Since the identified LANA-1 and ANG interacting common cellular proteins are hitherto unknown to KSHV and ANG biology, this offers a starting point for further analysis of their roles in KSHV biology which may lead to identification of potential therapeutic targets to control KSHV latency and associated malignancies.

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The Risks of Sports Specialization and Rapid Growth in Young Athletes

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Background: Literature regarding an independent relationship between sports specialization and risk of injury currently lacks, as does prospective research analyzing periods of measured rapid growth and risk of injury in young athletes. **Purpose:** 1. To determine if young athletes that specialize in a single sport have a greater risk of injury. 2. To determine if young athletes going through periods of rapid growth have a greater risk of injury. **Methods:** *Study Design*: Prospective Cohort Study. *Phase I:* Athletes presenting to clinic for evaluation of a sports-related injury were compared to those presenting for a sports physical. All consented participants completed a baseline survey evaluating sports participation, height and weight. Injured participants also completed an injury survey. The electronic medical record (EMR) was used to obtain retrospective height and weight data when possible. A sports specialization score was calculated, with 4 to 6 considered highly specialized, and 0 to 3 classified as less specialized. *Phase II:* Six-month interval follow-up will continue for the next three years, including height, weight, tanner stage, sports training changes and new injuries. The EMR will gather and validate information when possible. **Results:** From June through September 2010, 93 male and 63 females were recruited (ages 10-18, mean =13), with injured and uninjured groups balanced at baseline. Two relationships trended toward significance: the injured cohort spent more hours playing sports (19.8 hrs/wk vs 17.0 hrs/wk, p=0.09) and more hours in organized sports (11.0 hrs/wk vs 8.8 hrs/wk, p=0.07.) The mean specialization score was higher for the injured (3.49) versus the uninjured (2.75), (p=0.015). A significant difference existed between injured and uninjured groups stratified by sports specialization score (60.38% injured v 31.25% uninjured with sports specialization score 4-6, p=0.009). There was no difference in the mean growth rate (uninjured = 4.1 cm/yr, injured=4.7 cm/yr, p=0.41.) **Conclusions:** More highly specialized sport participation may be a risk for development of injury in young athletes. Training intensity (annual weekly hours in sports participation or in organized sports) may modify this risk. No relationship between rapid growth and development of injury was noted in this sample, but this relationship will be better evaluated during the prospective longitudinal portion of this study.

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Differential Expression of NELF Splice Variants in Human and Mouse Immortalized Gnrh Neurons

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Idiopathic hypogonadotropic hypogonadism (IHH) results from deficient gonadotropin-releasing hormone (GnRH) secretion or action. Kallmann Syndrome (KS) is due to migration failure of GnRH and olfactory neurons during development, resulting in IHH with anosmia. Nasal embryonic LHRH factor (NELF) is a nuclear protein that was differentially isolated from migratory GnRH neurons and mutations have been identified in IHH/KS. We previously found that although *Nelf* mRNA expression did not differ in mouse immortalized GnRH neuronal cells, NELF protein expression was greater in migratory compared with postmigratory cells. The goal of this study was to determine if mRNA/protein expression discordance was due to differential expression of splice variants (sv). We hypothesize that migratory GnRH neuronal cell lines (mouse GN11 and NLT; human FNCB4-hTERT) will have a different expression pattern of sv than postmigratory GT1-7 cells. PCR products from RT-PCR were cloned and individual colonies were analyzed by denaturing gradient gel electrophoresis (DGGE) and DNA sequencing. Preliminary results indicate six splice variants in mouse cells, and four splice variants in human cells. Human FNCB4-hTERT and all three immortalized mouse GnRH cells predominantly express variant 2 ranging from 72-77% in the cell lines. In addition, DGGE findings showed the prevalence of splice variants, which differed in specific cell types (pre-migratory and post migratory immortalized mouse GnRH neurons). Finally, we identified 3 novel splice variants in the immortalized mouse GnRH neuronal cells lines which have not been reported. The differential *Nelf* mRNA splice variant expression could play a role in GnRH neuron migration and/or function as well as in the human phenotype of IHH/KS.

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Relationship between Body Mass Index and Glucose Variability in Burn Patients

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Background: Severely burned patients often enter a hypermetabolic state that alters glucose consumption and insulin sensitivity. This may be due to a systemic inflammatory response and altered homeostatic set points. As such, glycemic control is a critical component of burn management. In addition to overall glucose levels, variability in glucose levels has been associated with poorer outcomes in critical care, trauma patients, as well as burn patients. However, glucose variability may be influenced by several factors including age, extent of injury, and body composition. Increased BMI is associated with altered hepatic glucose output, insulin sensitivity, and metabolic needs. Studies have shown that BMI may affect both glucose levels and variability in critically ill patients. With their post-trauma hypermetabolic state in mind, we asked whether BMI shows a relationship with glucose variability

in burn patients. **Methods:** We conducted a retrospective chart review of 214 patients admitted to the Loyola University Medical Center Burn Unit between 2008 and 2010. The patients included in this study were older than age 2, had a length of stay of at least 4 days, a burn injury of at least 5% total body surface area, and were injured within 24 hours of admission. Patients were included in this study regardless of glucose values at either extreme. Glucose variability was calculated as 1) the standard deviation in glucose levels, 2) the average magnitude of the differences between consecutive glucose readings, and 3) the percent of glucose readings outside of (80-150mg/dL). Plasma glucose values were monitored throughout the patient's stay as indicated for routine care and were recorded from both metabolic panels and bedside testing; multiple plasma glucose values within any half-hour interval were averaged. Body mass index was calculated using admission heights and weights and categorized by range (underweight, normal, overweight, obese). **Results:** Significant differences among the BMI ranges were found in: a) mean glucose values ($P<0.05$) with obese patients having highest values; b) the average magnitude of glucose swings ($P<0.05$) with underweight patients having the largest swings; c) the standard deviation in glucose values ($P<0.01$) with underweight and obese patients having the highest values; and d) the percentage of readings outside of the euglycemic range ($P<0.05$) with underweight and obese patients having the highest values. **Conclusions:** Our study suggests that there is an association between measures of glucose variability and BMI, with patients at either extreme of body mass tending to have higher variability.

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Prorenin Receptor Up-Regulation in the Brain Following Ischemic Stroke is Blunted by Angiotensin-(1-7)

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Stroke is a leading cause of death and of severe, long-term disability. Although the pathophysiology of stroke is multifactorial, growing evidence supports a major role of the renin-angiotensin system (RAS). Our previous data have demonstrated that angiotensin-(1-7) [Ang(1-7)], a breakdown product of Ang II, has cerebroprotective actions against ischemic stroke produced by endothelin-induced middle cerebral artery occlusion in rats. We have also demonstrated that Ang(1-7) has anti-inflammatory effects, blunting increases in the expression of iNOS, IL1α, IL6, CXCR4, and CD11b during stroke. We propose that this may be part of the mechanism by which Ang-(1-7) affords cerebroprotection during stroke, as much of the damage due to stroke is a result of excessive inflammation and an over-activation of microglia. It is believed that another member of the RAS, prorenin, has detrimental effects in both renal and cardiac fibrosis and other cardiovascular diseases. As blocking Ang II and enhancing Ang(1-7) signaling have both been demonstrated to have therapeutic potential in cardiac, renal, and CNS/stroke pathology, we propose that blocking the prorenin receptor (PRR) may also have a therapeutic effect in stroke. We now describe for the first time that PRR mRNA in the cerebral cortical infarct zone is up-regulated in stroke, both at 6 h and 24 h after stroke. Interestingly, this increase in PRR is significantly blunted at 24 h by 7 d of Ang(1-7) treatment. These data show for the first time that PRR is increased in the brain following stroke, and that Ang(1-7) can blunt this increase. More importantly, they demonstrate a novel therapeutic target for stroke. We are currently designing experiments to pharmacologically block PRR to study any potential neuroprotective effects.

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Prospective Evaluation of Risk Factors for Symptomatic Hemorrhoids

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Background: While hemorrhoids are common in the general population, risk factors have not been well defined in the literature. Our Study aims to prospectively assess risk factors, which predispose to symptomatic hemorrhoids in a cohort of patients evaluated by colorectal surgeons. **Patients and Methods:** A questionnaire encompassing over 80 factors possibly related to the development of hemorrhoids was completed by patients presenting with anal symptoms to the offices of five colorectal surgeons in an urban setting. The incidence of these risk factors in patients with symptomatic hemorrhoids was compared those without symptomatic hemorrhoids. **Results:** Of the 907 patients studied, 646 (71%) were diagnosed with hemorrhoids. Univariate analysis revealed a statistically significant difference between the hemorrhoid and control group in percentage of individuals over 40 years of age (63% *vs* 55%, p=0.02), income greater than $100,000 per year (50% *vs* 39%, p=0.01), individuals with a Bachelors degree or higher (78% *vs* 71%, p=0.02), family history of hemorrhoids (73% *vs* 63%, p=0.04), moderate or high intake of spicy food (41% *vs* 31%, p=0.006), consumption of 2 or more cups of tea or coffee daily (34% *vs* 27%, p=0.04), one or more episodes of diarrhea weekly (16% *vs* 25%, p=0.003), rushing to toilet with urgency to defecate (24% *vs* 35%, p=0.001), exertion on the toilet (17% *vs* 12%, p=0.04), and pain upon defecation (12% *vs* 19%, p=0.02). On multivariate analysis, the only statistically significant differences were in the percentage of individuals with family history of hemorrhoids (p=0.01; OR=2.0, 95%CI=1.2-3.3), and income over $100,000 per year (p=0.02; OR=1.9; 95%CI=1.1-3.2). **Conclusions:** This first reported prospective study of patients examined by colorectal surgeons revealed both family history and income greater than $100,000 per year were independently associated with symptomatic hemorrhoids.

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Muscarinic Control of Presynaptic Long-term Potentiation at Parallel Fiber-Purkinje Cell Synapses

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Background: The activation of muscarinic acetylcholine receptors has been shown to modulate synaptic plasticity in a variety of areas within the central nervous system. In certain areas, this modulation is accomplished via the release of endocannabinoids (CBs).

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Within the cerebellar cortex it has been shown that CB release can block the induction of presynaptic long-term potentiation (PF-LTP) at parallel fiber-Purkinje cell (PF-PC) synapses. Herein, we demonstrate that the application of the muscarinic agonist oxotremorine-m (oxo-m) during the tetanization period blocks the induction of PF-LTP, and that this effect is absent in the presence of the cannabinoid receptor antagonist AM251. In addition, this effect is abrogated by the guanosine nucleotide analogue GDP-β-S and the phospholipase C (PLC) inhibitor U-73122, suggesting that the CB release is mediated by a G-protein coupled receptor linked to PLC (G_q). There are five known isoforms of muscarinic acetylcholine receptors (M1-5), of which M1, M3, and M5 are thought to exert their actions at least partially through the activation of PLC. To investigate which receptors are triggering the release of CBs in this case, we employed the use of M1-M3 double knockout mice and found that oxo-m does not block PF-LTP in these mice, suggesting that it is either M1 or M3, or both, that mediates this effect.

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Evaluating the Roles of Chromatin Structure and Tup1p in Determining *Candida Albicans* Virulence Using Next-Generation DNA Sequencing

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Candida albicans is an opportunistic pathogen responsible for significant morbidity and mortality in immunocompromised patient populations. Iron availability is believed to play a key role in signaling or facilitating the commensal to pathogenic transition of *C. albicans infections.* Changes in gene expression, cell-surface antigens, and drug sensitivity have been reported in *C. albicans* populations as a response to alterations in environmental concentrations of iron. Genetic regulation by the conserved transcriptional co-repressor Tup1p is believed to help facilitate the iron-response *of C. albicans*, since a significant overlap exists between the regulatory networks affected by both iron availability and Tup1p regulation. Moreover, the well-documented inability of tup1 null mutants to enter hyphal (virulent) growth forms further testifies to the potential connection between iron response, Tup1 regulation, and virulence in *C. albicans*. Despite these findings, however, direct evidence linking Tup1 regulation to iron response is lacking. Our previous work on a Tup1 homolog in the baker's yeast model (*S. cerevisiae*) has demonstrated that Tup1 repression targets stress response genes and stabilizes chromatin structure at those promoter regions as a consequence of its repression mechanism. Therefore, to investigate the potential role of Tup1 repression in facilitating iron response, we are mapping changes in Tup1 localization and chromatin structure across the *C. albicans* genome following iron exposure using next-generation DNA sequencing technologies (ChIP-seq and MNase-seq). Identifying changes in chromatin structure and Tup1 localization in the context of well-defined iron response and tup1 repression gene regulatory networks will expand our understanding of Tup1's role in global iron regulation and *C. albicans* virulence.

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Stroma-Derived Exosomes Mediate Oncogenesis in Multiple Myeloma

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Bone marrow (BM)-derived mesenchymal stromal cells (MSCs) support multiple myeloma (MM) cell growth, but little is know about the putative mechanisms that may regulate the interaction between MM cells and the surrounding BM milieu. Exosomes are known to mediate cell-to-cell interaction. We therefore studied the role BM-MSCs-derived exosomes in supporting MM biology *in vivo* and *in vitro*. We found that primary normal and MM BM-MSCs release CD63+/CD81+ exosomes, as confirmed by electron microscopy and immunogold labeling. BM-MSCs DiD-labeled exosomes were transferred into MM cells, as shown by time-lapse confocal microscopy. This transfer was further validated in human MM cell lines incubated with murine (C57BL/6 miRNA-15a/16-$1^{-/-}$ and wild type) BM-MSCs-derived exosomes: qRT-PCR showed presence of murine miRNAs in human MM cells. The impact of BM-MSCs-derived exosomes on MM cell behavior *in vivo* was next evaluated. Normal and MM BM-MSCs-derived exosomes were loaded into tissue-engineered bones (TEB) with MM.1S-GFP+/Luc+ cells, and implanted s.c. into mice: MM cell homing and tumor growth were tested by using *in vivo* confocal microscopy and bioluminescence imaging. MM cells co-cultured with MM BM-MSC-derived exosomes induced rapid tumor growth at the site of TEB implant, and rapid dissemination to distant BM niches. In contrast, MM cells co-cultured with normal BM-MSC-derived exosomes led to minimal tumor growth and cell dissemination. We next explored the mechanisms by which exosomes may modulate MM biology: miRNA expression profiling was performed on exosomes isolated from normal and MM BM-MSCs. We found that miRNA15a is significantly lower in exosomes derived from BM-MSCs of MM patients ($P<.05$). We previously showed that miRNA15a shows lower expression in primary MM cells. We therefore sought to examine whether lack of transfer of tumor suppressor miRNA15a can modulate tumor growth and dissemination in MM. We overexpressed miRNA15a in BM-MSCs, and found inhibition of MM cell proliferation and adhesion to fibronectin. Exosomes isolated from BM-MSC-pre-miRNA15a-transfected cells both inhibited MM cell proliferation and adhesion. These findings demonstrate the existence of exosome-driven interactions between the BM milieu and MM cells, suggesting that exosomes constitute a novel mechanism for intercellular transfer of genetic information in the form of miRNAs in MM.

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Early Antibiotic Administration Reduces Methicillin-Resistant *Staphylococcus Aureus* Induced Cytokine Synthesis

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Introduction: Timely antibiotic administration in Methicillin-resistant *Staphylococcus aureus* (MRSA) sepsis improves clinical outcomes. We examined the effects of early and late antibiotic administration on MRSA-induced cytokine synthesis. Methods: Whole blood from healthy, human subjects was incubated *in vitro* with MRSA at various times and concentrations. The antibiotics vancomycin or linezolid were added at early (3 and 6 hours post-inoculation) or late time points (9 hours post-inoculation). To examine *in vivo* responses, wild-type C57/BL6 mice (n=8-10 per group) were given a single intraperitoneal (IP) injection of MRSA ($2.5x10^8$ colony-forming units (cfu) total). Mice were then treated with IP vancomycin (60 mg/kg) at early (2 and 8 hours post-infection) or late time points (8 and 14 hours post-infection). Twenty-four hours after infection mice were sacrificed and peripheral blood and peritoneal lavage fluid was collected. MRSA-induced cytokine responses (± antibiotics) were assessed by measuring interleukin (IL)-6, IL-8, and monocyte chemotactic protein-1 (MCP-1) levels by ELISA. Results: In human whole blood, MRSA induced the robust synthesis of the pro-inflammatory cytokines IL-6, IL-8, and MCP-1 in a time- and concentration-dependent manner. Over a 24-hour period, cytokine synthesis was detectable as early as 3-hours post-inoculation with $9x10^6$ cfu/mL MRSA. Independent of bacterial concentration, IL-6 and MCP-1 concentrations peaked at 12-hours post-infection, while IL-8 concentrations continued to increase up to 24-hours post-infection. Vancomycin administration (5X minimal inhibitory concentration, 5 μg/mL) within 6-hours of inoculation attenuated IL-6 (91% reduction), IL-8 (88% reduction), and MCP-1 (50% reduction) synthesis ($p<0.05$). If antibiotic administration was delayed >9 hours only IL-8 was significantly decreased (54% reduction vs. untreated MRSA controls, $p<0.05$). Similar results were seen with linezolid. Wild-type C57/BL6 mice infected with MRSA had a significant increase in MRSA bacterial counts compared to saline treated controls ($p<0.001$). Early vancomycin administration significantly decreased bacterial growth (93% decrease vs. untreated animals, $p<0.001$), while delayed administration did not significantly reduce bacterial growth. Consistent with our *in vitro* data, the early administration (within 2 hours of infection) of vancomycin in MRSA-infected mice reduced cytokine levels, compared to delayed administration (>8 hours post-infection). Conclusions: Early antibiotic administration inhibits MRSA growth and MRSA-induced pro-inflammatory cytokine responses both *in vitro* and *in vivo*. These data support clinical studies demonstrating the importance of timely antibiotic administration in MRSA sepsis.

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Ending Each Heartbeat: KCNE1 Slows the Voltage Sensors of KCNQ1

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Abstract: The heart is an electromechanical organ that couples myocardial action potentials (APs) to contraction. The timing and duration of APs are precisely tuned, and elongation of APs can lead to problematic repolarization of the ventricles and long QT syndrome (LQTS). The most prevalent form of the disease, LQT1, is due to mutation of the voltage-gated potassium channel KCNQ1. In complex with the β-subunit KCNE1, KCNQ1 α-subunits pass the late repolarizing cardiac current, I_{Ks}. Mutations in KCNE1 can also cause disease (LQT5). The difference in current obtained by expression of α-subunits alone versus complete I_{Ks} channel complexes is striking; KCNE1 slows activation and deactivation kinetics, suppresses inactivation, and increases single-channel conductance. Attempts to elucidate the mechanism of KCNE1-induced slowing of kinetics have come to discrepant conclusions. Two studies using chemical modifiers and cys-substitution in the KCNQ1 S4 supported one model where KCNE1 acts on voltage-sensors alone and another where only the activation-gate is affected. A fluorimetric study argued for effects on sensors and the gate (Osteen et al, PNAS 2010). To address the controversy, we used cut-open oocyte vaseline-gap clamp to record gating currents, ionic currents and perform site-directed, voltage-clamp fluorimetry (VCF). Gating currents show that voltage-sensor movement precedes pore opening in channels with only KCNQ1 α-subunits. VCF confirms this result. While ionic currents have a lag before activation, gating and fluorescence changes do not; this indicates a final concerted step after voltage sensors move before pore opening. In contrast, channels with KCNE1 show voltage-sensor movements with no lag that are ~20-fold slower and mirror slowing of ionic current activation. These findings are unlike those made by Osteen et al. We used a smaller dye inserted closer to S4 and measured ionic current and fluorescence simultaneously in the same cells. We observe a matching and expected shift in the I_{Ks} fluorescence-voltage and ionic current-voltage relationships of +50 mV with KCNE1 and do not see voltage-sensor movements at hyperpolarized voltages as they report. Both KCNQ1 and I_{Ks} channels have voltage-sensor kinetics almost as slow as ionic activation. Our simultaneous kinetic recordings of fluorescence and ionic currents support a model in which I_{Ks} channels are slowed purely by an effect of KCNE1 on the KCNQ1 voltage sensors. Support: HL105949, GM030376, Paul and Daisy Soros Fellowship for New Americans, UofC MSTP Frank Family Fellowship.

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Zbtb46 Expression Distinguishes Conventional Dendritic Cells and Their Committed Progenitors From Other Immune Lineages

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Distinguishing dendritic cells (DCs) from other cells of the mononuclear phagocyte system (MPS) is complicated by the shared expression of cell surface markers such as CD11c. We identified *Zbtb46* (*BTBD4*) as a novel transcription factor selectively expressed by conventional DCs (cDCs) and their committed progenitors, but not by plasmacytoid DCs (pDCs), monocytes, macrophages or other lymphoid or myeloid lineages. Using homologous recombination, we replaced the first coding exon of *Zbtb46* with GFP to inactivate the locus while allowing detection of *Zbtb46* expression. In *Zbtb46*$^{+/gfp}$ mice, GFP was expressed in committed cDC precursors (pre-cDCs) and lymphoid and peripheral cDCs, but not in pDCs, monocytes, macrophages, or other myeloid or lymphoid lineages. GFP^{+} precursors have restricted developmental potential, generating cDCs, but not pDCs, monocytes, or macrophages. *Zbtb46* overexpression in bone marrow progenitor cells selectively inhibits granulocyte potential and promoted cDC development. cDCs develop in *Zbtb46*$^{gfp/gfp}$ (*Zbtb46*-deficient) mice but maintain expression of G-CSF and LIF receptors normally downregulated in cDCs. Thus, *Zbtb46* may help enforce cDC identity by restricting DC responsiveness to non-DC growth factors, and may serve as a useful marker to identify rare cDC progenitors and to distinguish between cDCs and other MPS lineages.

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Aβ Derived from Alzheimer's or Cerebral Amyloid Angiopathy Brain Tissue Can Be Used to Seed NMR-Labeled, Biologically-Relevant Fibrils

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Alzheimer's Disease (AD) is the most common cause of dementia and affects 13% of the US population over 65. Most persons with AD also have the separate but related condition cerebral amyloid angiopathy (CAA). In contrast to the classic neuritic plaques of insoluble Aβ peptide which are diagnostic of AD, CAA displays purely vascular deposition of Aβ. Aβ comprises a group of peptides which have the ability to form fibrillar structures categorized as amyloid. Atomic-level structures of fibrils are of interest in understanding how to favor or disrupt fibril growth. Fibrils grown *in vitro* from synthetic Aβ display a variety of structures, dependent upon growth conditions and the sequence of Aβ used. However, neither the structures of Aβ fibrils which form in the brain, nor specific growth conditions which determine fibril structure *in vivo*, are well characterized. The structure of synthetic fibrils can be controlled by the addition of a preformed fibril 'seed' to non-aggregated Aβ. In particular, adding a seed to synthetic NMR-labeled Aβ produces fibrils which share the seed's structure and are suitable for ssNMR. Thus addition of brain-derived Aβ to labeled, non-aggregated Aβ results in formation of fibrils which share the structure(s) found in the brain and can be studied in detail using ssNMR. Traditionally, Aβ isolation from tissue has taken advantage of the relative stability of the fibrils to SDS and formic acid. Because these agents may compromise the structure of Aβ fibrils, we have pursued approaches which keep Aβ fibrils intact and representative of the entire population. In addition, because of the role environment may play in *in vivo* fibril formation, we are studying the structures of fibrils produced by using either vascular (CAA) or parenchymal (AD) Aβ as a seed. In this poster we describe our methods of isolation and present kinetic and electron micrograph results of seeding fibril growth by the addition of vascular and parenchymal Aβ isolates.

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Antigen-Dependent Control of Ntreg, Itreg and Tconv by TNFRSF25

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TNFRSF25 is a death-domain containing paralog of TNFR1 which is selectively expressed by lymphocytes and is activated by a monogamous ligand, TNFSF15 (TL1A). Historically, TNFRSF25 was described strictly as a pro-inflammatory receptor and shown to enhance CD4+ helper T cell polarization and effector function toward Th1, Th2 and Th17 lineages. This resulted in exacerbated disease pathology in experimental models of type 1 diabetes, multiple sclerosis, asthma and arthritis. Recently, we demonstrated that stimulation of TNFRSF25 using agonistic antibodies or TL1A-Ig fusion proteins resulted in profound and highly selective proliferation of FoxP3+ regulatory T cells (Treg) *in vivo* in the absence of additional exogenous stimuli. TNFR25-expanded Treg were functional and suppressed acute lung inflammation and allogeneic transplant rejection in mice. Treg expansion was dependent upon MHC II, IL-2 and Akt, but independent of mTORC1, CD28 and CTLA-4. The specificity of TNFR25 stimulation to Treg or FoxP3-negative conventional T cells (Tconv) is now shown to be dependent upon cognate antigen availability. This information provides an opportunity to guide selective proliferation of foreign antigen-specific iTreg together with self-antigen specific nTreg to control specific autoimmunity. Importantly, costimulation of TNFRSF25 together with antigen-specific vaccination also leads to simultaneous proliferation of iTreg and effector T cells with shared cognate antigen specificity. These data illustrate that TNFRSF25 performs an important role as a fulcrum between immunity and tolerance.

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UPEC Superinfection Leads to Chronic Cystitis in a Time- and Invasion- Dependent Manner during Murine UTI

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Urinary tract infections (UTI) are among the most common bacterial infections in women, with a lifetime risk approaching 50%. UTI caused by uropathogenic *E. coli* (UPEC) accounts for 80% of these infections and is often associated with high morbidity, recurrence, and chronicity. Studies in mouse models of UTI have shown that UPEC occupies both extracellular and intracellular niches within the bladder. During the acute phase of infection, UPEC invade the superficial epithelium and replicate into biofilm-like intracellular bacterial communities (IBCs). The outcomes of an acute infection range from resolution with or without accompanying quiescent intracellular reservoirs (QIRs) in the underlying tissue to chronic cystitis marked by persistent bacteriuria and inflammation throughout the lifetime of the mouse. High levels of IL-5, IL-6, G-CSF, and KC in the serum of C3H/HeN mice at 24 hours post infection (hpi) are prognostic of the development of persistent bacteriuria and chronic cystitis 4 weeks later. Thus, an early host checkpoint determines the fate of disease and interestingly, sexual intercourse frequency is a highly associated risk factor for UTI development. We hypothesized that superinfection impacts the host checkpoint and may mimic the risk factors associated with frequent sexual intercourse. Thus, we conducted superinfections in our murine model with WT and mutant UPEC and analyzed the impact on the development of persistent bacteriuria and chronic cystitis. We found that superinfecting C3H/HEN mice 1 or 6 hours after initial infection with WT UPEC dramatically increased the incidence of chronic cystitis, while a 24 hpi superinfection had no effect. We also found that invasion-deficient UPEC were unable to prime the bladder for the development of chronic cystitis upon superinfection with WT UPEC. Administration of LPS or heat killed UPEC was not sufficient to increase the predisposition for chronic cystitis. Superinfection of C3H/HeJ mice that lack TLR4 responsiveness had no effect. C57BL/6J mice are resistant to developing chronic cystitis. However, a 24 hpi superinfection surprisingly triggered the onset of chronic cystitis. These data show that a host response to intracellular bacteria during the first 24 hpi predisposes mice to develop chronic infection upon superinfection, suggesting that intercourse during periods of inflammation may predispose women to chronic UTI.

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Menopause Enhances Ischemic Induction of Dickkopf-1 and AD-Related Proteins in the Rat Hippocampus

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Dickkopf-1 (Dkk1) is a Wnt antagonist that is elevated in neurodegenerative disorders such as stroke and Alzheimer's disease (AD). Previously, our lab showed that physiological levels of 17 beta-estradiol (E2 or estrogen) were able to exert neuroprotection, in part, by suppressing the ischemia-induced elevation of neurodegenerative Dkk1 and activating the pro-survival Wnt/Beta-Catenin pathway. Here, we demonstrate that female rats subjected to global cerebral ischemia following a period of long-term estrogen deprivation (10 weeks post bilateral ovariectomy or reproductive senescence) display enhanced hippocampal induction of Dkk1 and the AD-related proteins beta-amyloid and hyperphosphorylated-tau. Furthermore, Dkk1 co-localized with neurons expressing the apoptotic marker TUNEL, which is consistent with its proposed neurodegenerative role. Importantly, these collective findings coincided with diminished expression of survivin, a key pro-survival protein product of the Wnt/Beta-Catenin pathway, and loss of estrogen neuroprotection in the hippocampus of long-term estrogen-deprived animals. As such, this study supports the "critical period hypothesis" of estrogen replacement and further suggests that *perimenopausal* estrogen therapy may prevent neurodegenerative changes in the hippocampus by maintaining favorable Wnt/Beta-Catenin signaling.

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Progressive Upregulation of Thrombomodulin, C-Reactive Protein and D-Dimer in Chronic Kidney Disease Versus End Stage Renal Disease can be used to Stratify Risk of Thrombotic Events

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Progression through the stages of Chronic Kidney Disease (CKD) and into End Stage Renal Disease (ESRD) is unpredictable and thus prognosis and risk are also difficult to predict. Cardiovascular events such as ACS and DVT are common complications in kidney disease; in fact 50% of mortality in ESRD is due to a cardiovascular event. Earlier and more sensitive indicators of CKD progression and cardiovascular risk must be established. Hematological studies suggest an elevated inflammatory and thrombotic state in kidney disease, however, the clinical utility of biomarkers have not been fully evaluated. This study evaluates the progression of CKD to ESRD through the pro-thrombotic markers Thrombomodulin (TM), C-Reactive Protein (CRP), and D-Dimer. 48 patients with CKD, 104 patients with ESRD, and 79 age matched control samples were evaluated. TM, CRP, and D-Dimer were measured simultaneously by Cerebral II biochip immunoassay technology (Randox Evidence Investigator, United Kingdom). Compared to controls, TM levels in CKD and ESRD were increased by 2.76 and 5.68 times respectively, CRP levels were increased 2.45 and 4.33 times in CKD and ESRD respectively, and D-Dimer levels in CKD and ESRD were increased by 2.07 and 3.63 times respectively. The upregulation of CRP, TM, and D-Dimer is consistent with the increased thrombotic and cardiovascular events seen in kidney disease. Moreover, the increased levels of these markers in ESRD compared to CKD can explain the higher risk of thromboembolic disease documented in ESRD. This study elucidates the progression of CKD to ESRD based on thrombotic state. Therefore, it is possible that biomarkers of inflammation and procoagulation can be used to evaluate the actual pathophysiological state and cardiovascular risk in kidney disease. Since cardiovascular disease is the leading cause of

death in this kidney disease, and it is thromboembolic diseases which threaten survival rather than reduced kidney function alone, the actual kidney disease stage may be less important than the patient's inflammatory/thrombotic state. Therefore, with the use of multiple biomarkers, clinicians may be able to identify more vulnerable patients within a kidney disease stage to more properly focus treatment through risk stratification. Future studies should evaluate whether thrombotic state markers should be used in conjunction with eGFR in evaluating kidney disease staging and overall thrombotic risk in this population.

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Loss of Autophagy Limits Metastasis in the 4T1 Mouse Model of Breast Cancer

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While autophagy has been shown to be tumor-suppressive in early stages of tumorigenesis, its role in metastatic progression is unclear. Autophagy has been shown to reduce reactive oxygen species generation and necrosis in primary tumors, two factors that promote metastasis. However, autophagy is also required for cell survival in response to nutrient deprivation and matrix detachment, conditions that disseminated tumor cells must survive in order to form metastases, and autophagy has been proposed to be required for survival of dormant disseminated tumor cells. How these opposing roles of autophagy affect metastasis *in vivo* remains unknown. Since both compounds that induce autophagy and compounds that inhibit autophagy are currently in phase I/II clinical trials for the treatment of solid tumors, evaluating the role(s) of autophagy in metastatic progression presents an opportunity to discover whether targeting this process therapeutically could specifically help prevent or limit metastasis, as well as elucidate conditions under which modulating autophagy to treat a primary tumor may actually enhance the metastatic potential of the tumor. To directly examine the role of autophagy in an *in vivo* model of breast tumor progression and metastasis, we used shRNA to knock down autophagy-regulatory gene *atg5* in the 4T1 mouse mammary tumor cell line, effectively blocking most autophagy in these cells. When injected into syngeneic Balb/c mice, primary tumor growth of 4T1 cells lacking *atg5* was largely unaffected, but the number and size of metastases to lung and liver was greatly reduced compared to parental and scrambled control cells. *In vitro*, the *atg5* knockdown cells also showed reduced invasion and migration in Boyden chamber assays. Intriguingly, we observed defects in tumor cell movement and cytoskeletal remodeling in the knockdown cells using digital time-lapse imaging. Immunofluorescence of cytoskeletal components showed increased actin stress fiber formation and altered focal adhesion structure, suggesting that the pro-metastatic role of autophagy that we observed *in vivo* could partially reflect a novel function for autophagy in tumor cell motility. In sum, we have demonstrated an *in vivo* pro-metastatic role for autophagy in this model system, as well as a novel and unexpected function for autophagy in tumor cell motility. This suggests that the role of autophagy in tumor progression, and specifically metastasis, is yet more complex than previously appreciated.

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HIV-1 Tat Protein Dysregulates Cerebrovascular Reactivity, Attenuating Cerebral Blood Flow to the Cortex

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Cerebral blood flow (CBF) has recently been shown to be dysregulated in persons with human immunodeficiency virus 1 (HIV-1), as reflected by decreased resting cortical perfusion and abnormal responses to metabolic demand. To understand the underlying pathophysiologic mechanisms, we used a small animal model for HIV-associated neuroinflammation (HIV Tat transgenic [Tat-tg] mice). Mice were exposed to transient hypercapnia in order to probe cerebrovascular reactivity (CVR), and CBF was then measured over the somatosensory cortex using Laser Doppler Flowmetry. The normal, expected increase in cortical flow was significantly attenuated in the Tat-tg animals compared to non-transgenic littermates (WT) - suggestive of decreased CVR to metabolic stimulus. Furthermore, direct examination of the pial arterioles using intravital microscopy revealed attenuated vascular dilation during exposure to carbon dioxide (CO_2). However, microvascular morphology and density of the cortical capillary bed were found to be indistinguishable in Tat-tg versus WT mice, as assessed by multiphoton imaging of coronal brain slices. In order to address whether perturbation of nitric oxide- cyclic guanosine monophosphate (NO-cGMP) vasodilatory pathway might contribute to observed effects, we exposed Tag-tg mice to a hypercapnic stimulus in the presence or absence of (i) gisadenafil, a phosphodiesterase 5 inhibitor (PDE_5-I) that prevents degradation of cGMP, and (ii) tetrahydrobiopterin (BH4), which is a limiting cofactor necessary for the production of NO. Gisadenafil largely restored normal CVR in Tat-tg mice exposed to a hypercapnic stimulus, whereas BH4 had little effect. These data suggest that HIV-associated neuroinflammation may dysregulate cerebral flow at least in part by perturbing normal vasodilatory responses within the CNS, through effects on cGMP metabolism and possibly PDE_5. Finally, our findings suggest that PDE_5 inhibitors may have therapeutic potential for restoring normal cerebral blood flow in persons with HIV-1.

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Neocytolysis is Associated with an Increase of Mitochondrial Content & Reactive Oxygen Species and Impaired Protection from ROS

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The increased RBC mass during hypoxia is overcorrected at the return to normoxia by destruction of the young RBCs, i.e. *neocytolysis*. The molecular basis of *neocytolysis* remains obscure. It has been suggested that *neocytolysis* is caused by very low erythropoietin levels; however RBCs lack a pathway for erythropoietin signaling. We hypothesize that the rapid changes of Hypoxia Inducible Factor (HIFs) levels that regulate many genes,

including erythropoietin, might account for *neocytolysis*. Two of these genes, i.e. (*BNIP3L/NIX*) which regulates mitochondrial autophagy and (*FOXO3a)* involved in erythroid differentiation and control of several genes` responses to reactive oxygen species (ROS), are attractive candidates for *neocytolysis* genesis. In hypoxia, a HIF-1 mediated increase of *BNIP3L* would reduce mitochondria by autophagy and decrease of *FOXO3a* would result in reduction of catalase, superoxide dismutase (SOD) and other enzymes. The RBCs generated during hypoxia would be susceptible to increased ROS which is generated by mitochondria. We created a mouse model to test this hypothesis and we evaluated the tested parameters before, at the end of hypoxic exposure, and during the normoxic return. Hematocrit increased by ~22% at the end of hypoxic exposure and decreased by ~10% at day 10 (D10) of post-hypoxic (PH) return, while at PH D4 there was a marked increase of mitochondrial content and ROS. In contrast, catalase activity was reduced at PH D0 and PH D4, but restored at PH D10. There was an inverse correlation between *Bnip3L* levels and mitochondrial contents in young RBCs ($CD71^+$/$Ter119^+$). *Foxo3a* mRNA decreased at PH D0 and D4 from normoxic levels. *Catalase* (a target gene of *Foxo3a*) transcript changes showed a similar pattern of changes in *Foxo3a* transcripts, and catalase enzyme activity. ROS in RBCs were markedly increased at PH D0 and D4. We demonstrate that increased mitochondrial ROS and impaired protection against oxidative damage by decreased catalase are major mechanisms of *neocytolysis*.

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Diabetes Mellitus Potentially Can Cause Diastolic Dysfunction

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Introduction: Diastolic dysfunction (DD) is observed in about 40% of patients with diabetes mellitus (DM). DM is associated with DD in epidemiological trails. Previously, cardiac oxidation and fibrosis have been associated with DD in animal models. We sought to determine whether these mechanisms occurred in the DM type 2 KK.CgAy mouse model. Furthermore, we sought to determine if advanced glycosylation end products (AGE), oxidative stress, and fibrosis may be involved causing DD. **Methods:** Echocardiography and single cell measures of cardiomyocytes were used to determine if wild type (C57/BL6J) and KK.CgAy mice had DD. Western blotting against dinitorphenylhydrozone derivatizatives detected oxidized protein levels (Oxyblot) and immunohistochemistry staining were performed to determine collagen levels. Sarcomere function of contraction and relaxation were assessed by IonOptix System under stimulation at 1.0Hz, 4ms duration under 37'C perfusion. **Results:** The DM type2 KK.CgAy mouse model shows DD by echocardiography. The ratio of E '/A' is significantly lower at KK.CgAy compared to C57 control mice (0.73±0.17 vs control 1.08±0.28, $P<0.05$). There was no difference in AGE, TGF-β and collagen levels when comparing control group to KK.CgAy mice. Oxidative modification of proteins was significantly increased in under 50KDa proteins group in KK.CgAy mice (3.44 ±0.09 fold, $P<0.01$). Resting sarcomere length was shorter in KK.CgAy mice (1.65±0.01 μm vs control 1.83±0.01 μm, $P<0.001$). Prolonged relaxation constant, tau value was significantly increased in KK.CgAy mice (0.13±0.014 μm vs control 0.08±0.005, $P<0.01$) indicating relaxation impairment of diasolic dysfunction in DM model. **Conclusion:** Our study indicates that DM can cause DD and that the molecular mechanism is associated with oxidative damage of proteins. Fibrosis and advanced glycosylation end products were not associated with DD in the DM type 2 KK.CgAy mouse model.

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Healthcare Utilization in OEF/OIF Veterans with Moderate to Severe Traumatic Brain Injury

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Traumatic brain injury (TBI) has become a common injury for veterans of Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF). Various reasons for the increase in TBI occurrence have been proposed, such as the use of explosives by insurgents, expanded screening programs, and increased efficacy of body armor, resulting in increased survivability of what were once fatal injuries. Estimates of the incidence of TBI amongst troops in OEF/OIF range from 15% to 22.8%, with a majority of these being mild TBI. Although a majority of TBIs are classified as mild, 92% of the cost is attributed to moderate to severe TBI, making classification of these injuries and subsequent health care utilization of special importance. This study is a retrospective chart review of 148 OEF/OIF veterans with moderate to severe TBI. Moderate and severe TBI are debilitating injuries, often exacting a serious physical and mental toll. Some of these injuries are severe enough to require constant monitoring or care. In order to identify veterans for this study ICD-9 codes were analyzed from FY 1999-2008 in order to compile a list of veterans with codes pertaining to TBI, which were then cross-referenced with the OEF/OIF roster to identify OEF/OIF veterans, of which there were 14,862. In order to limit the cohort to veterans with moderate to severe TBI, AIS-BR1 scores of 3-6 were reviewed. Only veterans that were wounded in battle and transitioned directly from DOD care to VA car were included in an effort to generate the most complete medical records, yielding a cohort of 148. Overall, this cohort was overwhelming male (96.6%), came from ground forces (87.8%) , and often suffered their TBI from blast injuries (60.1%). Patients with severe TBI had significantly longer initial hospital stays (75.07 days vs 47.83, p-value 0.03) , as well as more inpatient admissions (.80 admissions vs. 0.35, p-value 0.01) during the first year from discharge of the hospital. The most common ICD-9 codes for this cohort were rehabilitation related, with VA costs for the cohort averaging $154,794. Costs between the moderate and severe TBI groups were not significantly different.

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Novel Selective Autophagy Factors

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The autophagy pathway is an essential, phylogenetically conserved, component of host defense against viruses in eukaryotes. Selective autophagy is thought to play a specialized "cellular housekeeping" role, keeping the cytoplasm free of large substrates such as protein aggregates, damaged organelles (e.g. mitochondria), viruses, and other intracellular pathogens. Accumulation of these substrates has been linked to many diseases, including neurodegenerative disease, cancer, and aging as well as infectious diseases, but the molecular mechanisms of targeting substrates for autophagic destruction are not well understood. To address this, we performed an image-based, genome-wide siRNA screen to identify host factors involved in selective autophagy of viruses (virophagy). Ninety-six of the 141 hits from the primary virophagy screen were also hits in a secondary screen for selective autophagy of mitochondria (mitophagy), suggesting that there are broad links between virophagy and other forms of selective autophagy involved in "cellular housekeeping." The screen results enabled us to identify the E3-ubiquitin (Ub) ligase SMURF1 as a novel player in virophagy of genetically diverse viruses and in mitophagy. We found that SMURF1 interacted with viral nucleocapsids and colocalized with damaged mitochondria and that SMURF1 dependent selective autophagy proceeds via a novel, Ub ligase-independent, manner. Strikingly, three hits in our screen (FANCC, FANCF, and FANCL) were members of the Fanconi anemia (FA) core complex that repairs reactive oxygen species (ROS)-mediated DNA damage. FA is a rare, autosomal recessive disorder in which mutations in any of 13 FA genes lead to chromosomal instability, bone marrow (BM) failure, leukemia, and solid tumors, leading to premature death. FANCC has additional, DNA repair-independent, cytoprotective functions (such as suppression of ROS levels and proinflammatory cytokine production) that are critical in the pathogenesis of FA and overlap with recently discovered functions of autophagy. We have confirmed that FANCC is a novel host virophagy and mitophagy factor in primary MEFs and in patient-derived fibroblasts, and that defective mitophagy in FANCC-deficient cells is associated with elevated levels of mitochondrial ROS (mtROS). Furthermore, FANCC deficiency restores neurovirulence of a herpes simplex virus (HSV)-1 mutant that is unable to inhibit autophagy. Together, these data validate our screen, set the stage for a more detailed exploration of the mechanisms of selective autophagy, and may lead to the identification of novel therapeutic targets for the treatment of Fanconi Anemia.

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Irgm1, the Murine Homolog of a Crohn Disease Susceptibility Gene, Regulates Intestinal Epithelial Function

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Crohn disease (CD) is an inflammatory bowel disease (IBD) that is characterized by ulcerative lesions in the distal small intestine and colon. There is a significant but complex genetic contribution to CD susceptibility, and genome-wide association studies have identified 71 risk alleles to date. One of these alleles is associated with the gene *IRGM*. *IRGM,* along with its murine homolog *Irgm1,* have previously been shown to be required for autophagy-mediated clearance of intracellular bacteria and protozoans in immune cells. Using the *Irgm1* knockout mouse model, we are exploring the role of this gene specifically in the regulation of the intestinal epithelium, the site of IBD-associated inflammation. We first re-derived the *Irgm1*$^{-/-}$ mouse into an enhanced barrier facility. These mice are healthy and breed at Mendelian ratios, although they are approximately 20% smaller in mass than their wild-type (WT) C57BL/6 littermates. They do not develop spontaneous colitis. In response to challenge with dextran sodium sulfate, a toxic compound used in inflammatory models of IBD, *Irgm1*$^{-/-}$ mice do not differ in weight loss or intestinal pathology compared to WT mice. However, we have found two key abnormalities in the intestinal epithelium of mice deficient in *Irgm1*. First, intestinal epithelium of *Irgm1*$^{-/-}$ mice experiences increased proliferation and turnover compared to WT mice. Nevertheless, *Irgm1*$^{-/-}$ mice do not have a heightened rate of intestinal tumour development (seen in preliminary investigations in mice up to 5 months of age). Second, *Irgm1*$^{-/-}$ mice have enlarged goblet cells throughout the intestinal tract. Goblet cells secrete mucus into the lumen of the intestine, which can provide a barrier against microbes. Interestingly, research in our lab suggests that autophagy proteins may be important in goblet cell function. We are currently investigating whether Irgm1 is associated with autophagy in the intestinal epithelium and if this is the mechanism by which it affects the morphology and homeostasis of this complex tissue. These findings will impart novel insights into the role of the epithelium itself in the pathogenesis of CD and potentially offer new targets for therapy.

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Prolactin Receptor-Mediated Internalization of Imaging Agents Detects Epithelial Ovarian Cancer with High Specificity and Sensitivity

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Epithelial ovarian cancer (EOC) has the highest mortality rate of all gynecologic malignant tumors. Diagnosis of epithelial ovarian cancer (EOC) presents two main challenges. The first challenge is detecting low volume (< 1 g) and early stage (≤ stage II) masses to prevent rapid progression to late stages and ultimately death.

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The second challenge is differentiating malignant from benign tissue to avoid costly and invasive surgeries (19.5 surgeries are required to find 1 cancer even with multiple screenings). First-line diagnostic tests such as ultrasound and serum marker tests (e.g. CA-125) aid in diagnosis but they lack the sensitivity and specificity required to overcome both challenges. Magnetic resonance imaging (MRI), a second-line diagnostic aided by gadolinium based contrast agents (CAs), offers higher resolution pictures for classifying indeterminate ovarian masses. But as currently practiced, MRI still lacks the sensitivity and specificity required to alter patient outcomes. In this work we develop a new paradigm for EOC diagnosis that targets the prolactin receptor (PRLR) - a cell surface tyrosine kinase receptor that is over-expressed in moderate to high levels on > 98% of epithelial ovarian cancers. Upon binding of native ligands to PRLR, the ligand:PRLR complex is internalized by cells. By conjugating gadolinium-chelates, molecules normally used as contrast agents diagnostically, to human placental lactogen (hPL), a native ligand of PRLR, we show that MRI becomes highly sensitive and specific for detecting PRLR (+) tumors in a nude mouse model of EOC. We further establish the adaptability of this approach for fluorescence-based imaging techniques using an hPL conjugated Cy5.5 dye. We conclude that molecular imaging of PRLR with hPL-conjugated imaging agents can address the current challenges that limit EOC diagnosis.

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Long-Term Propranolol Use in Severely Burned Pediatric Patients Reduces the Inflammatory Response

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Background: Severe burn injury results in a hypermetabolic response that persists up to 2 years post burn. The purpose of this study was to determine the effects of long-term administration of propranolol on the inflammatory response as a part of a large prospective randomized single-center controlled trial. **Patients and Methods:** 196 pediatric patients with burns > 30% total body surface area were prospectively enrolled in the study. During their acute admission patients were randomized to receive either placebo (n-86) or propranolol (n-108) at a dose of 4mg/kg/day for 12 months post burn. Changes in inflammatory mediators were measured at patient discharge (~3m) and at 6, 9, 12, 18 and 24 months post burn. Statistical analysis used Tukey t test or ANOVA followed by Bonferroni correction with significance accepted at $P < 0.05$. **Results:** Long-term propranolol treatment significantly reduced the inflammatory response. Significant decreases in IL-6, IL-8, IL-10, GM-CSF, and MCP-1 were found in patients treated with propranolol. **Conclusions:** Propranolol treatment for 12 months following injury ameliorates the hyperinflammatory response in severely burned pediatric patients. IL-6 and MCP-1 reductions may indicate a reduction in post-burn insulin resistance; follow-up studies will be needed to determine the correlation. **Funding Sources:** This study was supported by grants from the National Institute for Disabilities and Rehabilitation Research (H133A070026 and H133A70019), the National Institutes of Health (P50-GM60338, R01-HD049471, R01-GM56687-11S1, and T32-GM8256), and Shriners Hospitals for Children (84080, 8510, 8740, 8741, 8660, 9145, 8760, 71009, and 71008). CCF is an ITS Career Development Scholar supported, in part, by NIH KL2RR029875 and NIH UL1RR029876 (CTSA). This study is registered at clinicaltrials.gov, NCT00675714.

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Perirhinal Cortex Bridges Time in Trace Eyeblink Conditioning

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Hippocampal function is required for acquisition, but not post-consolidation (long-term) performance, of trace eyeblink conditioning (tEBC), a time-bridging associative learning task. However, the contributions of parahippocampal areas during this time course are not well understood. Perirhinal cortex (PR) has been shown to be essential for familiarity and some recognition tasks, and some neurons in PR show persistent spiking in vitro, a possible mechanism for bridging time between stimuli. We explored the hypothesis that time bridging in tEBC may be mediated by perirhinal cortex. We lesioned bilateral PR in F344xBN rats prior to acquisition or post-consolidation (30 days) of tEBC (conditioned stimulus = tone, unconditioned stimulus = airpuff to eye, trace = 250msec) and found an acquisition deficit (percent conditioned responses) in PR-lesioned animals as compared to shams. Preliminary data indicate post-consolidation impairment as well, suggesting that PR maintains a role in tEBC at a time when hippocampus is no longer required for task performance. Preliminary data from in vivo single-unit recordings in PR and connected nodes show persistent spiking during the trace period of tEBC, suggesting that PR can contribute to bridging time between stimuli.

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Glucocorticoid Receptor-Mediated Cell Survival Following Androgen Receptor (Ar) Blockade In Castrate-Resistant Prostate Cancer (Crpc)

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Introduction: Sustained AR signaling is a hallmark in the development of CRPC. Potent AR inhibitors, such as MDV3100, are effective in CRPC, however, resistance is inevitable. We have shown that following androgen deprivation therapy for PC, tumor glucocorticoid receptor (GR) expression is increased. The GR and AR are known to share similar DNA binding sequences and to regulate several common target genes, including the gene encoding the anti-apoptotic protein serum/glucocorticoid regulated kinase 1 (SGK1). We hypothesized that increased GR expression might compensate for diminished AR signaling, thereby facilitating resistance to AR inhibitors. **Methods:** CWR-22RV1 (22RV1) and LAPC4 prostate cancer cell lines were grown *in vitro* with combinations of the AR antagonist MDV3100 (10μM), synthetic androgen R1881 (1nM), GR agonist dexamethasone (Dex, 1μM or 100nM) the GR inhibitor mifepristone (Mif, 100nM) and/or the SGK1 kinase inhibitor GSK650394 (1μM). **Results:** Following MDV3100 treatment, GR expression increased in both cell lines.

There was relative protection from MDV3100-associated cell death in association with increased GR activity. For LAPC4 cells, cell survival compared to R1881 control increased significantly with Dex treatment (66% vs. 47%). For 22RV1, Dex treatment also increased cell survival despite MDV3100 (151% vs. 51% without Dex). When the GR antagonist Mif was added with MDV3100, there was a synergistic impairment of survival. For LAPC4 cells, compared to R1881+Dex control, there was no change in survival with the addition of Mif alone, but a significant reduction (52% vs. 26%) in viable cells with MDV3100+Mif vs. MDV3100 alone. To test if SGK1 was required for GR-mediated protection from cell death following AR inhibition, the cell lines were exposed to the SGK1 inhibitor GSK650394. Cell survival with was impaired following treatment with the SGK1 inhibitor. For LAPC4, relative to control, survival was 58% with MDV3100+Dex versus only 19% with the addition of GSK650394. Results with the 22RV1 cells were similar. **Conclusions:** These data suggest that increased GR expression and activity antagonizes the effectiveness of AR inhibitors by promoting tumor cell survival. Furthermore, GR-induced SGK1 appears to be a downstream mediator of this effect. Further studies are underway to test this hypothesis. If supported, inhibiting the GR/SGK1 pathway may be a useful adjuvant approach to blocking AR activity and tumor cell survival in CRPC.

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The Promoter of Metallothionein 1X Is Epigenetically Regulated and Directs Its Over-Expression in Metal-Induced Bladder Cancer

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Metallothioneins are cysteine-rich, small metal-binding proteins that are expressed in different human tissues. The over-expression of metallothioneins has been documented in different types of bladder cancer. Arsenite and Cadmium are bladder carcinogens that have been used to transform the normal human urothelial cell line, UROtsa. Studies by this lab have confirmed the over-expression of MT1X in metal-transformed cell lines and bladder cancer. RNA isolation and RT-PCR has shown the significant over-expression of the arsenite-transformed cell line, when compared to the normal-parental and cadmium-transformed cell lines. In this study, chromatin immunoprecipitation analysis was used to assess the binding of transcription factors and to assess histone tail modifications in normal and metal-transformed UROtsa cells. Results show increase in Acetyl H4 and H3K4me3 modifications and a decrease in H3K27me3 modifications in the metal-responsive region of the MT-1X promoter of the arsenite-transformed cell line. Additionally, several transcription factors such as p300, NF-1, Sp1 & LBP1 showed an increased binding to this region. Our data implicate the arsenite-transformation of the UROtsa cell line in the epigenetic modifications of the promoter of the MT1X gene that has resulted in its over-expression in that particular cell line.

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Enriching Donor Satellite Cells with $\alpha 2\delta 1$ Promotes Differentiation

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Cell transplants into skeletal muscle of patients with muscular dystrophy are limited by donor cell attachment, migration, and survival in the host tissue. In animal models, despite HLA matching and a reduction of the host's immune response, few donor cells are retained in the host muscle. Enriching cells for a surface marker that enhances ability of the cell to attach, migrate, and survive will result in improved cell retention. The purpose of this study was to determine whether $\alpha 2\delta 1$-enriched primary satellite cells were better candidates for cell transplant than satellite cells without this surface marker. The $\alpha 2\delta 1$ subunit is part of the L-type calcium channel but appears earlier than the other subunits. We isolated satellite cells from the hind limb muscles of neonatal mice and separated four subpopulations of cells based on the presence or absence of $\alpha 2\delta 1$ and a marker of quiescence, CD34, by fluorescence activated cell sort (FACS). These sorted satellite cells were either plated or injected into irradiated hindlimbs of mdx mice. *In vitro*, satellite cells enriched with $\alpha 2\delta 1$ survived in heat deactivated media past day 19, while there was no evidence of attachment or survival of cells without $\alpha 2\delta 1$. In addition, cells that were positive for $\alpha 2\delta 1$ and negative for CD34 demonstrated the most robust myogenesis out of all four subpopulations. Enhanced myogenesis of this subpopulation was determined by morphology, the pattern of expression of myogenic transcription factors, and the development of excitation-contraction coupling as demonstrated by the presence of L-type calcium currents and calcium transients. As the data relate to differentiation and survival, these results suggest that cells enriched with $\alpha 2\delta 1$ and without CD34 will demonstrate greater cell retention and force generation after satellite cell transplant. *In vivo*, donor cells from all four subpopulations survive for one week after graft, but with distinct trends of fusion, new myotube formation, and distribution in the host muscle.
Supported by MDA

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Lymphotoxin Reduces Commensal Diversity to Enable Diet-Induced Obesity

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Commensal microbes exist in a state of symbiosis with host immunity and are essential for weight gain in diet induced obesity (DIO). Lymphotoxin α (LTα), a key molecule in mucosal defense, has been linked to obesity. We report that mice lacking LTα, LTβ, and LTβ-receptor (LTβR) resist DIO. The microbial communities of LTβR$^{+/-}$ mice and LTβR$^{-/-}$ mice differed on normal chow (NCD) and after high fat diet (HFD). The microbial community of LTβR$^{+/-}$ mice was less diverse after HFD, a hallmark of the "obese microbiome" in humans. On the contrary, LTβR$^{-/-}$ animals maintained similarly diverse communities to their NCD counterparts while on HFD. A specific microbe, Segmented Filamentous Bacteria (SFB), was cleared after HFD in LTβR$^{+/-}$ but not LTβR$^{-/-}$ mice and served as

a marker of diversity loss. Transplantation of cecal contents from LTβR$^{-/-}$ animals conferred less weight gain than that of LTβR$^{+/-}$ mice to WT germ free mice, demonstrating a causal relationship between differing microbiota and weight gain. Housing LTβR$^{-/-}$ mice with their LTβR$^{+/-}$ siblings rescued weight gain, demonstrating horizontal transmissibility of the obese phenotype. HFD induced IL-23, an SFB associated cytokine, within the colon, but this induction was absent in LTβR$^{-/-}$ mice. Mice deficient in IL-23 (p19$^{-/-}$) and the downstream cytokine IL-22 (RORγt$^{-/-}$) also resisted DIO. Our data suggests that HFD induced inflammation reduces commensal diversity to enable weight gain, which may underlie the pathogenesis of obesity.

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Investigation Of Nomethiazoles As A Treatment For Alzheimer's Disease

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Background: Alzheimer's Disease is multifactorial and elicits diverse neurological and psychiatric abnormalities, and therefore presents a unique challenge in drug development. Nomethiazoles, a novel drug class developed in Dr. Thatcher's lab, are chimeric compounds that retain the neuroprotective GABA-potentiating activity of chlormethiazole (CMZ) and add the ability to activate CREB via NO-mimetic actions. Elaboration of the drug class proceeded under NIA support using a functional screening method that was also used for discovery of mechanisms of action. In primary neuronal culture models of excitotoxicity and amyloid-induced toxicity, neuroprotection correlated to the GABA-potentiating ability of methiazole backbones. In electrophysiological investigation in hippocampal slices, reversal of deficits in LTP in 3xTg mice were found to be mediated through an NO/cGMP mechanism. Additionally, in long-term studies in the 3xTg mouse model of AD, procognitive effect was demonstrated in STPA, a behavioral model of memory, which neatly paralleled increases in levels of pCREB. Finally, these neurorestorative effects *in vivo* also led to a decrease in levels of Abetaβ, tau and the neuroinflammatory/apoptotic marker TNF-α.

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HAART-Experienced Women with Detectable Plasma Viral Load Continue To Have Genital Tract HIV-1 RNA Shedding Despite Changing Treatment Regimens in a Clinical Care Program

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Objective: We longitudinally assess plasma viral load (PVL) and genital tract viral load (GTVL) among HIV-infected women changing highly active antiretroviral therapy (HAART) because of detectable PVL on current HAART regimens. **Methods:** Paired plasma and GT HIV-1 RNA were measured prospectively over 3 years. Longitudinal analyses examined rates of GT HIV-1 RNA shedding and the association with PVL. **Results:** Sixteen women contributed a total of 205 study visits of whom all had detectable PVL and 69% had detectable GT HIV-1 RNA at baseline. Half of the women were changed to new HAART regimens with 3 or more active antiretroviral drugs. The probability of having detectable plasma HIV-1 RNA ≥30 days after changing HAART regimens was 0.56 (95% CI: 0.37 to 0.74). Fourteen women (88%) had detectable PVL on a follow-up visit at least 30 or 60 days after changing HAART regimens; and 12 women (75%) had detectable GTVL on a follow-up at least 30 or 60 days after changing HAART regimens. When PVL was undetectable, shedding occurred in at least one of the three genital subcompartments at 13% of visits; when PVL was detectable, shedding occurred in at least one of three subcompartments at 50% of visits. **Conclusions:** A significant proportion of extensively treatment-experienced women continue to have detectable virus in both the plasma and genital tract following a change in treatment regimens, highlighting the difficulty of viral suppression in this patient population. Further longitudinal studies are needed to better understand the interaction between genital tract and plasma viral load following virological failure.

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Impact of Point-of-Care Testing and a Physician in Triage on the Timely Assessment of Patients with Chest Pain

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Objective: This study assessed the impact of placing a physician in triage and the utilization of troponin point-of-care testing (POCT) on timeliness of management for patients presenting to the emergency department (ED) with chest pain. **Methods:** A retrospective chart review was performed. For 220 patients presenting to the ED with chest pain, we compared time intervals to clinical events between periods before and after the implementation of physician in triage and troponin POCT. The primary endpoints were time from arrival to EKG and time from arrival to first troponin assay result. Secondary endpoints included time from arrival to disposition and length of stay. **Results:** There was a mean decrease of 26 minutes (95% confidence interval [CI] 7 to 45 minutes) in the time from arrival to troponin assay result after implementation of POCT and physician in triage. Time from arrival to disposition decreased 100 minutes (95% CI 41 to 160 minutes) and length of stay was decreased by 188 minutes (95% CI 107 to 269 minutes). There was no significant difference in the time from arrival to EKG between groups. **Conclusions:** The use of POCT and a physician in triage significantly decreased the time from arrival to first troponin result but did not significantly effect on the time from arrival to EKG. Significant decreases in time to disposition and length of stay were also observed, suggesting these measures impact more than the first few minutes after a patient presents to the ED.

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Cancer Exome Analysis Reveals a T Cell Dependent Mechanism of Cancer Immunoediting

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Cancer immunoediting, the process whereby the immune system controls tumor outgrowth and shapes tumor immunogenicity, is comprised of three phases: elimination, equilibrium and escape. Although many immune components that participate in this process are known, its underlying mechanisms remain poorly defined. A central tenet of cancer immunoediting is that T cell recognition of tumor antigens drives the immunologic destruction or sculpting of a developing cancer. However, our current understanding of tumor antigens comes largely from analyses of cancers that develop in immunocompetent hosts and thus may have already been edited. Little is known about the antigens expressed in nascent tumor cells, whether they are sufficient to induce protective anti-tumor immune responses or whether their expression is modulated by the immune system. Here, using massively parallel sequencing, we characterize expressed mutations in highly immunogenic methylcholanthrene-induced sarcomas derived from immunodeficient *Rag2*$^{-/-}$ mice which phenotypically resemble nascent primary tumor cells. Employing class I prediction algorithms, we identify mutant spectrin-β2 as a potential rejection antigen of the d42m1 sarcoma and validate this prediction by conventional antigen expression cloning and detection. We also demonstrate that cancer immunoediting of d42m1 occurs via a T cell-dependent immunoselection process that promotes outgrowth of pre-existing tumor cell clones lacking highly antigenic mutant spectrin-β2 and other potential strong antigens. These results demonstrate that the strong immunogenicity of an unedited tumor can be ascribed to expression of highly antigenic mutant proteins and show that outgrowth of tumor cells that lack these strong antigens via a T cell-dependent immunoselection process represents one mechanism of cancer immunoediting. They also point to the future potential benefits that cancer genome and transcriptome analyses may bring to the fields of tumor immunology and cancer immunotherapy.

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The Loop 5 Element Structurally and Kinetically Coordinates Dimers of the Human Kinesin-5, Eg5

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Eg5 is a homotetrameric kinesin-5 motor protein that generates outward force on the overlapping, antiparallel microtubules (MTs) of the mitotic spindle. Upon binding a MT, an Eg5 dimer releases one ADP molecule, undergoes a slow (~0.5 s^{-1}) isomerization, and finally releases a second ADP, adopting a tightly MT-bound, nucleotide-free (APO) conformation. This conformation precedes ATP binding and stepping. Here, we use mutagenesis, steady-state and presteady-state kinetics, motility assays, and electron paramagnetic resonance (EPR) spectroscopy to examine Eg5 monomers and dimers as they bind MTs and initiate stepping. We demonstrate that a critical element of Eg5, loop 5 (L5), accelerates ADP release during the initial MT-binding event. Furthermore, our EPR data show that L5 mediates the slow isomerization by preventing Eg5 dimer heads from binding the MT until they release ADP. Finally, we find that Eg5 having a 7-residue deletion within L5 can still hydrolyze ATP and move along MTs, suggesting that L5 is not required to accelerate subsequent steps of the motor along the MT. Taken together, these properties of L5 explain the kinetic effects of L5-directed inhibition on Eg5 activity and may direct further interventions targeting Eg5 activity.

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Manipulation of the RANKL/RANK/OPG Axis Using Structure-Based Design and Yeast Surface Display

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The interaction between Receptor Activator of NF-κB Ligand (RANKL) and its receptor RANK is key to the differentiation and function of the osteoclast, the sole bone resorbing cell. Osteoprotegerin (OPG), a soluble homodimer, acts as a decoy receptor for RANKL. RANK and OPG contain four similar cysteine-rich repeat domain (CRD) regions, but OPG binds RANKL with greater affinity and markedly different kinetics. An imbalance in the RANKL/RANK/OPG axis with decreased OPG and/or increased RANKL is associated with diseases that favor bone loss, including osteoporosis. To explore the structural basis of RANK versus OPG binding to RANKL, we designed 34 RANKL mutants. Our initial screens have identified six candidate RANKL mutants, all of which contain CD loop insertions, which retain variable levels of OPG binding (including normal) but show no detectable binding to RANK. These findings are also in accordance with the co-crystal structures which demonstrate a dramatic rearrangement of the RANKL CD loop when bound to RANK versus OPG and may explain the observed differences in affinity despite the similar footprints of the interfaces. Using similar methods, we were not able to design mutations that abolish binding to OPG while leaving binding to RANK intact. Therefore, we have established a yeast surface display system to screen libraries of randomly mutated RANKL proteins and select for these properties. RANKL is expressed on the surface of yeast fused to the C-terminus of an inducible yeast mating protein, Aga2p. Using flow cytometry, we can detect a dose-dependent increase in OPG and RANK binding to surface displayed wild-type RANKL, indicating functionality of our system. After multiple rounds of sorting randomly mutagenized RANKL libraries, we appear to have enriched for RANKL mutants that have lost the ability to bind OPG while still retaining the ability to bind

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RANK. We are now in the process of screening individual clones to identify novel mutations in RANKL with these properties. Once identified, we will analyze recombinant proteins using biophysical and biological read-outs of function.

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Expression Levels of the DNA Repair Enzyme, UNG, Predict Lung Cancer Sensitivity to Pemetrexed

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Uracil misincorporation is a direct consequence of TS inhibition by the multi-target antifolate pemetrexed. The DNA base excision repair (BER) enzyme Uracil DNA glycosylase (UNG) is the major glycosylase responsible for the removal of misincorporated genomic uracil. We have previously illustrated that human colon cancer cells (DLD1) with engineered stable somatic knockout of UNG expression are hypersensitive to pemetrexed owing to the cytotoxicity of genomic uracil accumulation. Here, we have examined the relationship between the expression of UNG and the therapeutic effect of pemetrexed in a small panel of lung cancer cell lines (n~10). UNG expression levels were measured by western blot (for protein) and RT-PCR (for mRNA). Results indicated that UNG mRNA expression is well correlated with protein expression in all cell lines. Moreover, there was wide range of distribution of UNG levels in the lung cancer cell lines evaluated. UNG cutting assay and AP site measurements confirm variations in UNG expression result in varied potential for BER activation and subsequent AP site formation. Analysis of tissue array data in Oncomine indicated a similar range of UNG expression in primary human lung cancer samples. To determine the role of this variation in UNG expression in pemetrexed cytotoxicity, we performed colony survival studies. In general, cell lines with higher UNG expression were more resistant to pemetrexed cytotoxicity. As an example, H460 cells have a 4-fold lower UNG protein expression and are 5-fold more sensitive to pemetrexed than H1975 cells (H460 IC_{50} = 200nM; H1975 IC_{50} = >1000nM). To confirm differential sensitivity was dependent upon changes in UNG expression status, we engineered stable knockdown of UNG expression using shRNA in the moderately resistant cell line, A549. Colony survival experiments indicate that decreased UNG expression results in increased sensitivity to pemetrexed growth arrest and cell killing (IC_{50} A549 parental = 400nM; IC_{50}A549 sh-UNG = 20nM). A clinical relevance to these data is suggested by Oncomine analysis that revealed higher median levels of UNG expression in notoriously pemetrexed resistant lung cancer types - small cell lung cancer and squamous cell lung cancer (1.5-fold greater than adenocarcinoma, $p<0.005$). In sum, our findings illustrate differentially expression of the BER protein UNG in lung cancer cells, which may have particular impact on the therapeutic impact of pemetrexed and perhaps other antifolates. These data motivate future evaluation of UNG as a predictor of clinical response to pemetrexed in lung cancer patients.

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NK Cells in HMPV Infection

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Human metapneumovirus (HMPV), a paramyxovirus discovered in 2001, is a major cause of acute respiratory tract disease in children worldwide. This negative-sense, single-stranded RNA virus also affects elderly and immunocompromised individuals. No vaccine for the virus is currently available. Relatively little is known about the role of Natural Killer (NK) cells in HMPV infection. NK cells are known to have a wide variety of functions during infections in general, including the release of IFNγ, perforin, and granzyme, as well as the release of cytokines that modulate T cell activity. We hypothesize that NK cells have an important role in enhancing both the innate and adaptive immune responses against HMPV infection. To test this hypothesis, NK cells will be depleted in a mouse model to determine the effects on HMPV replication and pathogenesis. Three monoclonal antibodies (NKp46, CD49, and NK1.1) were tested using flow cytometry at different concentrations to determine the most effective antibody concentration to detect NK cells. A protocol using mAb PK136 (anti-NK1.1) to deplete NK cells in vivo was optimized. After depleting NK cells with PK136 antibody and infecting the animals with HMPV (control animals will be administered isotype control antibody), viral titer will be measured by infectious focus assay and quantitative PCR. Lung histopathology and weight loss post-infection will be used as measures of disease severity. The role of NK cells in modifying adaptive B- and T-cell immune responses will be examined by measuring cytokine expression, antibody production, and epitope-specific CD8+ T cell number and function. To test the hypothesis that antibody-dependent cell-mediated cytotoxicity (ADCC) is important for HMPV clearance, FcR-deficient mice will be used to compare differences in duration of disease, lung histopathology, cytokine production, and viral titer between knockout and wild-type mice. These findings will help elucidate features of the immune response against HMPV, and may also provide new ideas for potential treatments in the future.

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Adenosine Prevents Epileptogenesis via an Epigenetic Mechanism

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Epigenetic changes, including hypermethylation of DNA, contribute to the development of epilepsy; consequently, therapeutic approaches that reverse those changes might prevent epileptogenesis. Here we identify a novel epigenetic function of the brain's endogenous anticonvulsant adenosine. We demonstrate that adenosine induces hypomethylation of DNA in rodents via biochemical interference with the transmethylation pathway. A bioengineered silk-based brain implant was used to transiently deliver a defined dose of 250ng of adenosine per day over 10

days to the brains of epileptic rats. This therapeutic intervention reversed the DNA-hypermethylation seen in the epileptic brain, prevented the sprouting of mossy fibers in the hippocampal formation, and prevented the progression of epilepsy for at least 3 months. Thus, therapeutic delivery of adenosine to the brain prevents epileptogenesis by interference with DNA methylation.

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The Melanoma Stem Cell Marker ABCB5 Is Functionally Required For Efficient Tumor Formation, Chemoresistance, and Cancer Stem Cell Maintenance

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Melanoma remains a disease with relatively few beneficial treatments currently available, for late-stage patients. The ATP-binding cassette (ABC) transporter ABCB5 has recently been identified as a cell-surface marker for malignant melanoma initiating cells (MMICs), opening a new paradigm for investigation of this aggressive cancer. We have previously demonstrated that these ABCB5$^+$ MMICs can evade the host immune system, and also express VEGFR-1, required for efficient tumor formation. However, the possibility that ABCB5 itself plays a functional role in melanoma tumorigenic growth and progression has not been investigated, until now.

We found that inhibiting ABCB5 in human melanoma cell lines, with shRNA or inhibitory antibody, resulted in significantly increased expression of the whey acidic protein 4-disulphide core domain 1 (WFDC1/ps20) tumor suppressor, which can act as a WNT signaling inhibitor. We also observed a concomitant decrease in interleukin 8 (IL8), a known driver of melanoma that is both a putative downstream WNT target and also associated with dacarbazine (DTIC) chemoresistance. Consistent with changes in these gene levels, and impaired WNT signaling, we showed that ABCB5 shRNA stable knockdown melanoma cells had a significantly reduced ability to form xenograft tumors in immunodeficient mice, and displayed a more differentiated pigmented phenotype when forming tumors *in vivo*. Furthermore, ABCB5 shRNA cells were chemosensitized to DTIC *in vitro*, implying a secondary role for ABCB5 as a mediator of DTIC chemoresistance. Finally, we demonstrate that inhibiting the ABCB5-regulated IL8 pathway results in disruption of the ABCB5$^+$/ABCB5$^-$ cell equilibrium, consistent with a reduction in MMICs.

Altogether these data suggest a model whereby ABCB5 is functionally required for efficient melanoma progression, by driving WNT signaling and maintaining the MMIC component, explaining its specific presence on the MMIC. These findings represent one of the first demonstrations of malignancy-relevant multifunctional roles for a cancer stem cell marker.

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Intersectin1 Links Amyloidogenic Process in Down syndrome & Alzheimers Disease

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Late-life manifestations of Down Syndrome (DS) are of increasing importance, as DS patients now survive well into middle age. By the age of 35, all DS patients exhibit Alzheimers Disease (AD)-like neuropathology and twenty percent are affected clinically. This comorbidity is attributed to trisomy of AD-related genes within the Down Syndrome Critical Region (DSCR) of chromosome 21. Despite its prominent role in AD-pathogenesis, triplication Amyloid Precursor Protein (APP), a DSCR protein, produces a restricted phenotype compared to the AD-like phenotype produced by triplication of the entire DSCR. This suggests that additional DSCR genes are required for AD development; the scaffold protein intersectin1 (ITSN1) is a leading candidate. ITSN1 is the most highly induced transcript in non-DS cases of AD. In DS, ITSN is overexpressed and differentially expressed in the brains of DS patients with and without AD. Here, we expand these findings to show that ITSN overexpression is sufficient to increase the processing of wildtype and AD-linked variants of APP. We also show that loss of ITSN drastically reduces APP processing in cell-based models. This effect is paralleled in the brains of ITSN-null mice: ITSN1 null mice exhibit a two fold increase in full length APP compared to littermates with a single copy of ITSN1, suggesting that ITSN is an important regulator of *in vivo* APP processing. On going experiments will determine whether ITSN mediates alpha, beta, and/or secretase cleavage of APP in modes of AD and DS. Lastly, we show that ITSN1 overexpression increases the activation of c-Jun-N-terminal kinase (JNK), which is known to augment pathogenic forms of APP processing. Based on these data, we hypothesize that ITSN1 overexpression, in DS and AD, increases the processing of APP into neurotoxic cleavage products (e.g. Beta-Amyloid) through JNK.

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Fibroblast Stiffness Sensation during Spreading Is Conducted by the Lamellipodium and is Myosin-Independent

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Multicelluar organisms require cells to adhere to their extracellular matrix (ECM) and to other cells. External mechanical cues that organize coordinated cellular behaviors have been hypothesized to play important roles in development, tissue maintenance, and disease progression. More specifically, the transformed behavior of isolated mammalian cells in culture and cancer cells *in vivo* have been associated with the stiffness of their ECM. We demonstrate that mammalian fibroblasts on fibronectin alter their terminal spread area bimodally as a function of ECM stiffness. These cells largely fall into two regimes: on soft ECM's cells often remain rounded and do not spread well or polarize, and on stiff ECM's cells spread well, polarize, and gain the appearance of cells plated

on glass or plastic. The transition region between our two regimes is ~2kPa and loosely corresponds to proposed transitions for focal adhesion maturation and actin bundling. We also found evidence that this transition is not mediated by nonmuscle myosin II (NMMII) motor contractility, and that by modulating lamellipodial protrusion through manipulation of Rac1 signaling, we can impair fibroblasts' ability to spread in a stiffness-dependent way on fibronectin ECM's. These results have lead us to the conclusions that cellular mechanosensation during spreading is independent of NMMII contractility and dependent on Rac1-mediated actin polymerization in the lamellipodium. This hypothesis suggests that nascent adhesions signal through Rac1 in the lamellipodium to modulate spread area as a function of ECM stiffness.

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Development of Atherosclerosis in a Population Endemic for Geohelminths

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Background Atherosclerosis is a marker of cardiovascular disease which is characterized by chronic inflammation and dyslipidemia. As chronic helminth infection is associated with lower nutritional status and anti-inflammatory response, we hypothesize that helminth infections can protect against the development of atherosclerosis. **Methods** A cross-sectional study was performed in Flores, Indonesia, an area highly endemic for geohelminths. Stool samples were collected from 1040 participants and diagnosed for *Trichuris trichiura* by microscopy and for *Ascaris lumbricoides* and *Necator americanus* by PCR. In the same group we measured information on atherosclerosis risk factors such as body mass index (BMI), waist to hip ratio (WHR), blood pressure (BP), fasting blood glucose, lipid levels, high sensitive C-reactive protein (Hs-CRP) level and LPS stimulated cytokines (tumor necrosis factor(TNF)-α and interleukin(IL)-10. A subset of 426 elderly adults participated in intima media thickness (IMT) measurement. **Findings** Here we found no direct association between helminth infection and IMT. We did find a negative association between BMI, WHR, and systolic BP with *N. Americanus* infection. *A. lumbricoides* infection was negatively associated with diastolic BP and *T. trichiura* infection was negatively associated with total cholesterol (TC) and high density lipoprotein (HDL-c). The effect of *N. Americanus* on BP was dependent on age, with a clear effect in the elderly. The effects were also shown on TC, light density lipoprotein, and TC/HDL-c ratio. Similar association was found for *T. trichiura* infection and CRP. **Conclusion** This over age cross-sectional study presents unique evidence that helminth infections were negatively associated with risk factors atherosclerosis risk factors and might protect the host against the development of atherosclerosis.

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IL-18-Primed 'Helper' Natural Killer Cells Mediate the Attraction and Activation of Dendritic Cells, Promoting the Accumulation of Type-1 Effector T Cells for Anti-Cancer Immunity

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The chemokine-driven association of immune cells critical to the initiation, propagation, and execution of immune responses is essential for the development of effective immunity against cancer. We have previously shown that natural killer (NK) cells can be primed by IL-18 to a unique 'helper' phenotype capable of promoting dendritic cell (DC) activation and potent DC-mediated type-1 immune responses most effective against cancer. Here, we further demonstrate that such IL-18-treated 'helper' NK cells are selectively primed for high expression of immature DC (iDC)-attracting chemokines, including CCL3 and CCL4, upon subsequent exposure to tumor cells, type I interferons, or other secondary signals. These 'helper' NK cells potently attract iDCs in a CCR5-dependent mechanism, situating them for efficient NK cell-mediated activation. We further describe the unique ability of these 'helper' NK cells to induce high DC production of CXCR3 and CCR5 ligands, facilitating the recruitment of type-1 effector T (T_{eff}) cells critical for tumor rejection. Furthermore, using immediate *ex vivo* treatment of cells derived from the malignant ascites of patients with advanced ovarian cancer, we demonstrate the therapeutic potential for using helper NK cell-inducing stimulatory factors to enhance T_{eff} cell-recruiting chemokines directly within the human tumor environment. This study demonstrates for the first time the unique chemokine regulation of 'helper' versus 'effector' pathways of human NK cell differentiation in the initiation and propagation of DC-mediated immune responses. These data further provide rationale for the therapeutic use of properly-activated NK cells in promoting type-1 immune responses for enhanced anti-cancer immunity.

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Adolescents Are At Greater Risk for Cocaine Addiction than Adults

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Adolescence is a period of heightened propensity to develop cocaine addiction in humans. We first show that midbrain dopamine neurons are more active in adolescent rats compared with adults using in vivo extracellular recording. Given that elevated activity of dopamine neurons is associated with elevated propensity to self-administer cocaine in rats, we tested whether the period of adolescence is associated with higher liability to self-administer cocaine relative to adulthood.

Adolescent (postnatal day 42) and adult (~postnatal day 88) rats were compared for cocaine addiction liability using self-administration. We show that adolescent rats are more likely to acquire self-administration of cocaine than adults, show greater motivation and escalate in intake more readily. These findings

parallel the greater addiction liability observed in human adolescents relative to adults.
The next question to ask is: are they more likely to *remain* addicted to cocaine compared with adults? In one experiment we asked how punishment associated with drug intake affected subsequent drug use. To do this, cocaine was delivered together with an electric footshock during one of the self-administration sessions. Cocaine intake is suppressed for both ages on the day of the electric footshock. However, the next day adolescents resume cocaine taking whereas adults do not. In a second experiment we examined how onset of cocaine use affects stress-induced relapse later on. Here, following self-administration, rats withdrew from cocaine in their home cages for over 40 days, a time when the adolescents had grown into adults. We then subjected rats to stressful experiences known to trigger relapse: electric footshock or injection of the stress hormone corticosterone. Both stressors trigger relapse, but this is far more pronounced if onset of cocaine use occurred during adolescence vs. adulthood. Our results indicate that cocaine use during adolescence has critical consequences. Our research is the first to offer scientific evidence that when all opportunities to take drugs are equal, biology alone makes adolescents more likely to use cocaine compared to adults. We also show that adolescents do not refrain from taking cocaine after punishment, and they are more likely to relapse in response to a stressor later on in life than adults. These results have broad implications both for the attempts to steer adolescents away from drug use using punishments as well as for preventing relapse. Together our results indicate that adolescents are at greater risk for cocaine addiction than adults.

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Thyroid Hormone Induces Mouse Pancreatic Acinar Cell Proliferation, Polyploidization and Metaplasia *in Vivo*

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Background and Aim: Thyroid hormones are major regulators of development, growth and metabolism. Thyroid hormone regulates dramatic remodeling of the Xenopus pancreas during metamorphosis, inducing transient dedifferentiation of acinar cells to a progenitor phenotype, followed by redifferentiation to mature ducts and acini. We hypothesized that adult mouse pancreatic acinar cells stimulated by thyroid hormone would demonstrate characteristics of pancreatic progenitor cells. **Methods:** Male C57Bl/6 mice were injected with thyroid hormone (T3, 4 mg/kg i.p. daily for 4 or 7 days) and sacrificed on day 5, 8 or 10. Bromodeoxyuridine (BrdU) was administered 2 hrs prior to sacrifice. Markers of proliferation (Ki67, BrdU), phenotype (amylase, ductal cytokeratins, insulin and glucagon) and differentiation (Sox9, Pdx1) were labeled by immunofluorescence in formalin-fixed paraffin sections. Ploidy was estimated using in situ hybridization for the Y chromosome. **Results:** T3 dramatically increased acinar cell proliferation (BrdU and Ki-67) and modestly increased acinar cell ploidy when compared with controls. Zymogen granule content and amylase labeling were markedly reduced in T3-treated mice, but there was no evidence of inflammation or edema. Ductal cytokeratins and the progenitor marker Sox9 were upregulated in acinar cells after T3. Insulin and glucagon remained restricted to islets. **Conclusion:** Increases in circulating thyroid hormone induce acinar cell proliferation and metaplasia toward a ductal/progenitor, but not endocrine phenotype. The reversibility and long-term effects of T3-induced changes are currently under investigation.

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Long Non-Coding RNAS Are Dysregulated in Autism Post-Mortem Prefrontal Cortex and Cerebellum

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The genetics underlying autism spectrum disorders (ASD) are heterogeneous and the regulation of implicated loci is likely complex. Long non-coding RNAs (lncRNAs) have recently been implicated in a number of fundamental gene regulatory events, but their roles in ASD have not been studied comprehensively. To determine if dysregulated expression of lncRNAs may play a role in the molecular pathogenesis of ASD, we profiled over 33,000 annotated lncRNAs from ASD patient post-mortem brain tissue (prefrontal cortex and cerebellum) using custom microarrays. We detected 225 lncRNAs that were differentially expressed between ASD and control samples (FC>2 and p<.05). The majority of differentially expressed lncRNAs were from intergenic regions (~60%), antisense to protein-coding loci (~15%), or intronic (~10%). Almost 50% of differentially expressed lncRNAs map to within 50 kilobases of an annotated gene, and the ontologies of these nearby genes implicated two functions: cerebral cortex cell migration and targets of mir-103/107 (FDR q-values <.05). In parallel, we assessed for transcriptional differences of all known protein-coding mRNAs. Because our samples from both brain regions were from the same set of patients, we had the ability to compare intra-individual differences in expression within the brain. In agreement with recent large transcriptomics studies, we observed more transcriptional homogeneity within ASD brains. In light of this, then, we were intrigued to find that the number of lncRNAs differentially expressed within controls brains was also much greater than lncRNAs differentially expressed within autism brains (1375 versus 236, respectively). Moreover, unsupervised hierarchical clustering analysis of all samples was the same by lncRNAs and mRNA loci. Our results suggest an interplay between lncRNAs and protein-coding loci may contribute to ASD genomics, and/or that a fundamental defect in genome-wide transcriptional regulation underlies ASD pathology. This work provides comprehensive evidence that the lncRNA component of the transcriptome deserves further attention in ASD.

Oral Presentations

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ADAM10 Regulates Plasma Cell Differentiation

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A disintegrin and metalloproteinase 10 (ADAM10) is a zinc dependent proteinase related to matrix metalloproteinases. ADAM10 has emerged as a key regulator of cellular processes by cleaving and shedding extracellular domains of multiple transmembrane receptors and ligands. In particular, ADAM10 has been identified as a key regulator of lymphocyte development. Studies revealed that ADAM10 is a key initiator of Notch2 signaling and in the absence of ADAM10, marginal zone B cell development was impaired. Moreover, deletion of B-cell expressed ADAM10 resulted in changes in lymphoid tissue architecture and impaired germinal center formation. In this study, we generated a mouse line in which ADAM10 is deleted specifically in B cell following class switching, taking advantage of the cre-lox system (IgG1-cre)($ADAM10^{fl/fl}IgG1^{+}$). Our results demonstrate that deletion of ADAM10 following class switching does not alter lymphoid architecture; moreover, germinal centers form normally. Despite these findings, antibody responses to T-dependent and T-independent antigens are impaired $ADAM10^{fl/fl}IgG1^{+}$ mice, implicating ADAM10 in post-germinal center B cell differentiation. Surprisingly, plasma cell numbers defined as $B220^{-}CD138^{+}$ cells, were normal in spleen, bone marrow and peripheral blood of $ADAM^{fl/fl}IgG1^{+}$ when compared to littermate controls. We then decided to examine whether, despite normal CD138 expression, ADAM10-deficient plasma cells showed changes in gene expression. The transcriptional regulator Bcl6 is essential for germinal center formation but must be downregulated for plasma cell differentiation to occur as it directly inhibits plasma cell transcriptional program. While the level of *Irf4* and *Prdm1,* plasma cell-lineage transcription factors, were relatively normal, Bcl6 levels were 7-fold higher in ADAM10-deficient plasma cells. These results demonstrate that ADAM10 is required for proper downregulation of Bcl6 and as a result, plasma cell differentiation and antibody secretion is abnormal in $ADAM10^{fl/fl}IgG1^{+}$ mice. We have thus identified ADAM10 as a regulator of plasma cell differentiation/

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Identification of Serum- and Glucocorticoid-Inducible Kinase 1 (SGK1) as a Novel Regulator of Effector T Cell Differentiation

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T cell differentiation is determined by various signals in the immune microenvironment, including costimulatory ligands, cytokines, and the availability of nutrients. We have shown that the mammalian target of rapamycin (mTOR) signaling pathway is a master regulator of this differentiation process. mTOR can associate with 2 distinct protein complexes (TORC1 and TORC2) to drive the selective differentiation of various effector T cell subsets. In CD4+ T cells, loss of TORC1 leads to an inability to adopt a Th1 phenotype, while the loss of TORC2 leads to an inability to adopt a Th2 phenotype. In an effort to define the critical downstream targets of mTOR in T cells, we have identified the serum- and glucocorticoid-inducible kinase 1 (SGK1) as a TORC2 target responsible for directing T cell differentiation. We generated mice in which SGK1 is specifically deleted in T cells (*T-SGK1-/-*). Our studies demonstrate that CD4+ T cells from *T-SGK1-/-* mice preferentially adopt a Th1 phenotype *in vitro,* characterized by high expression of Tbet and production of IFNγ. Remarkably, CD4+ T cells from *T-SGK1-/-* mice adopt a Th1 phenotype even when skewed under Th2 conditions with IL-4. Mechanistically, SGK1 appears to regulate E3-ligase mediated destruction of JunB and the ability of TCF-1 to inhibit IFNγ. In an *in vivo* model of allergic airway hypersensitivity, *T-SGK1-/-* mice are resistant to Th2-mediated disease. Instead, these mice inappropriately mount a Th1 response, characterized by IFNγ production by lung lymphocytes and high titers of antigen-specific IgG2a. Altogether, these results suggest that SGK1 is responsible for repressing Th1 differentiation and promoting Th2 differentiation. In addition to its role in CD4+ T cell differentiation, our preliminary studies suggest that SGK1 plays an important role in CD8+ T cell proliferation and differentiation into effector and memory subsets. CD8+ T cells lacking SGK1 produce more effector cytokines and proliferate faster than WT cells *in vitro*. Additionally, in an *in vivo* model of viral infection, CD8+ T cells from *T-SGK1-/-* mice express higher levels of the memory marker CD127. Altogether, these results suggest that CD8+ T cells from *T-SGK1-/-* mice differentiate into superior effector and memory T cells. In summary, our findings reveal a novel role for SGK1 as an essential component of the mTOR pathway that guides differentiation of both CD4+ and CD8+ T cells. Targeting SGK1, therefore, may be a useful strategy for fine-tuning immune responses by manipulating T cell differentiation.

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Mutations in CARD14 Lead to Psoriasis and Psoriatic Arthritis

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Psoriasis is a common, immune-mediated, genetic disorder of the skin associated with arthritis in up to 30% of patients. Previously, we localized PSORS2 (psoriasis susceptibility locus 2) to chromosome 17q25.3-qter following a genome-wide linkage scan in a family of European origin with multiple cases of psoriasis and psoriatic arthritis. Linkage to PSORS2 was also observed in a multiply affected psoriasis family from Taiwan. With genomic capture and DNA sequencing, we identified unique gain-of-function mutations, p.Gly117Ser/c.349G>A and c.349+5G>A, in caspase recruitment domain protein 14 (CARD14) segregating with psoriasis in the two families. A *de novo* mutation in CARD14 (p.Glu138Ala) was detected in a sporadic, early-onset, generalized pustular psoriasis patient. To identify other psoriasis-associated mutations in CARD14, we re-sequenced additional psoriasis and psoriatic arthritis cases and controls. We identified multiple novel, missense mutations and determined their distribution in 7 independent psoriasis case/control cohorts (>6,000 cases and >4,000 controls). CARD14 harbored an excess of rare variants in cases versus controls (burden test p-value = 0.0015, variable threshold test p-value = 0.0053). Some variants were only seen

in a single case and included putative pathogenic mutations (p.Glu142Lys, p.Glu142Gly) and the generalized pustular psoriasis mutation p.Glu138Ala. The p.Gly117Ser mutation was present at a frequency of 0.0005 in cases of European ancestry. CARD14 activates nuclear factor kappa B (NF-kB), and we determined the effects of mutations on NF-kB activation. Variants led to a range of NF-kB activities, with putative pathogenic mutations, including p.Gly117Ser and p.Glu138Ala, leading to levels >3-fold higher than wildtype CARD14. Two variants (p.His171Asn and p.Arg179His) required additional stimulation with TNF-α to achieve significant increases in NF-kB levels. Immunostaining of skin confirmed the presence of CARD14 in epidermal keratinocytes and revealed an atypical distribution in psoriatic skin. We conclude that, following an inflammatory signal, altered regulation of the pathways mediated by *CARD14* in skin keratinocytes and immune cells leads to psoriasis. These studies increase our understanding of the pathogenesis of psoriasis and illustrate the challenges faced in identifying disease-causing genetic variants in common disease.

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Investigating the Therapeutic Effect of Rhes Suppression in a Mouse Model of Huntington's Disease

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Background: Huntington's Disease (HD) is a lethal neurodegenerative disease caused by expanded polyglutamine repeats in the huntingtin (HTT) protein. Currently there is no treatment to stop the disease progression. Despite the ubiquitous expression of mutant HTT (mHTT), the brain undergoes topologically selective degeneration with the greatest amount of early cell loss in the striatum. Recent cell culture studies reveal that a striatum enriched protein, Rhes, interacts with mHTT, and this interaction may account for the selective vulnerability of the striatum in HD. The objective of this study is to implement the *in vitro* findings of Rhes' interaction with mHtt to an *in vivo* investigation of the therapeutic potential of a Rhes targeting therapy. **Methods**: We designed inhibitory RNAs targeting murine Rhes using our established artificial miRNA expression system. Two inhibitory RNAs (miRhes2 and miRhes4) that silence endogenous Rhes expression at variable levels were next tested in the BacHD mouse model. BacHD mice express full-length mHTT and behavior deficits starting at 2 months of age. To assess the potential of Rhes suppression therapy on preventing disease progression, 4-month-old BacHD mice and wild-type littermates were injected with miRhes2, miRhes4, and miControl (scrambled miRNA). The behavioral phenotypes of the mice were measured using the accelerating rotarod test and Light-Dark Box Test at 4, 8, 12, and 14 months of age. The effects of Rhes suppression on striatal volumes were monitored longitudinally by magnetic resonance imaging (MRI) at 9 and 12 months of age. **Results**: Four months post-injection, HD mice treated with miRhes2 and miRhes4 showed improved motor activity and anxiety-like behaviors compared to HD mice treated with the control vector. Notably, Rhes suppression improved ambulatory distance, ambulatory time, and jump counts in HD mice. These partial disease modifying effects diminished with age. Longitudinal MRI measurements of brain volumes showed that Rhes suppression was well-tolerated 5 months post-injection. However, 8 months post-injection, striatal tissue loss was enhanced in HD mice which were treated with miRhes4, the most potent of the Rhes-targeting constructs. miRhes2 and control treated HD mice were indistinguishable from each other and showed reduced striatal loss relative to miRhes4-treated HD mice. **Conclusion**: In this proof-of-concept study, we demonstrate the feasibility of RNAi mediated Rhes suppression for eliciting short term improvements in symptomatic HD mice. Our findings also raise caution that prolonged Rhes suppression is detrimental.

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Prospective Assessment of Determinants of ApoB, ApoA1, and the ApoB/ApoA1 Ratio in 797 Healthy Schoolgirls, Studied From Mean Ages 10 To 19 Years: The Cincinnati National Growth And Health Study.

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Background: Focusing on pediatric determinants of ApoA1, ApoB and the ApoB/ApoA1 ratio should facilitate primary approaches to prevention of coronary heart disease (CHD), given strong, independent association of the ApoB/ApoA1 ratio to CHD. **Objectives:** Over 9 years, we prospectively assessed determinants of apolipoproteins B, A1 and the ApoB/ApoA1 ratio in 402 black and 395 white healthy Cincinnati schoolgirls from mean ages 10 to 19. **Materials/Methods:** ApoB was measured at mean ages 10, 12, 14, 16 and 19 and ApoA1 at mean ages 12, 14, 16, and 19. At age 14, total and free testosterone and sex hormone binding globulin (SHBG) were measured. Menstrual cyclicity < and ≥ 42 days was assessed from ages 14 to 19. **Results:** From ages 12 to 19, by virtue of lower ApoB, higher ApoA1, lower ApoB/ApoA1, and lower triglyceride (TG), black girls had a more favorable apolipoprotein-TG risk factor profile, which, compared to white girls, should be less associated with adult atherosclerosis. Our first novel finding was that menstrual cyclicity ≥ 42 days from ages 14-19, associated with hyperandrogenism, was independently associated with increasing ApoB/ApoA1 ratios. At age 14, SHBG was inversely correlated with the ApoB/ApoA1 ratio in both white and black girls. In black girls, SHBG at age 14 was inversely correlated with ApoB and the ApoB/ApoA1 ratio at ages 14 and 17-19, and was positively correlated with ApoA1 at ages 14 and 19. At age 14, in black girls, bottom quintile SHBG was associated with higher ApoB, lower ApoA1, and a lower ApoB/ApoA1 ratio. At age 19, the ApoB/ApoA1 ratio was higher in black girls with top quintile insulin levels, congruent with higher ApoB/ApoA1 ratios in black girls with annual reports of menstrual cycles ≥ 42 days, since hyperinsulinemia, low SHBG, and oligomenorrhea commonly co-aggregate, particularly in polycystic ovary syndrome (PCOS). From ages 14-19, BMI and TG were independently positively associated with ApoB. Menstrual cyclicity ≥ 42 days, metabolic syndrome, BMI, and triglyceride were independently positively associated with increasing ApoB/ApoA1

ratios, while black race was negatively associated. **Conclusion:** Our findings have implications for understanding the cardiometabolic issues associated with oligomenorrhea and PCOS. Our findings of a atherogenic association of low SHBG and menstrual delay in adolescence with increasing ApoB/ApoA1 foretells findings in adult women.
Supported in part by NIH- HL55025, 48941, HL52911 and HL66430, and Lipoprotein Research Fund.

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Targeting Tumor Antigen HER2/Neu to Receptor Treml4 on CD8α^+ Dendritic Cells *In Situ* Induces Anti-Tumor Immunity

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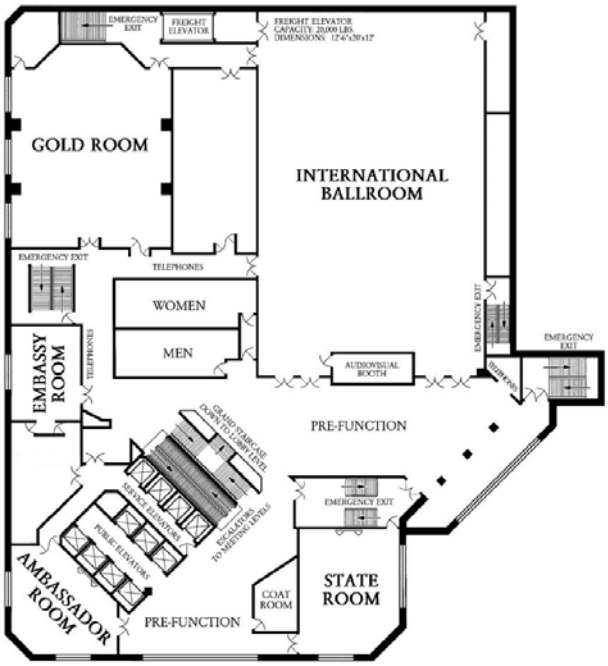
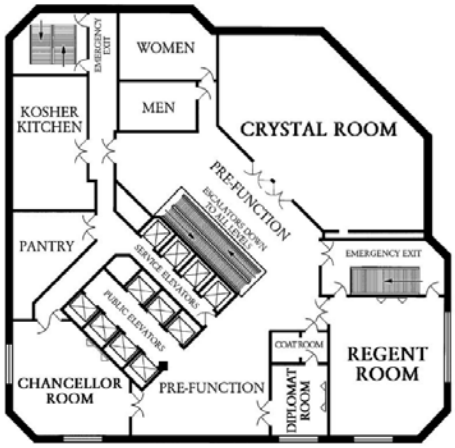
Cancer vaccines are biologics that elicit a specific immune response to eradicate tumor cells. Our strategy is to target dendritic cell (DC) receptors, such as DEC-205/CD205 and Treml4, with hybrid monoclonal antibodies (mAbs) fused to cancer antigens. Presentation of the cancer antigen to DCs initiates specific CD8 cytotoxic T-cell and T_H1 type CD4 immune responses aimed to boost adaptive immunity against clearing the tumor. In this study, we developed a hybrid mAb carrying the breast cancer antigen HER2/neu to target Treml4, a receptor highly expressed on the CD8α^+ DC subset that is superior in cross presentation to elicit specific CD8 cytotoxicity, as well as to subsets of splenic resident macrophages. Targeting HER2/neu to the receptor Treml4 in vivo induced specific CD4 and CD8 T-cell responses in mice of different genetic backgrounds. Of note was that we injected the mAb with adjuvant poly ICLC to stimulate maturation of DCs, without which tolerance to the tumor antigen would be induced. Using a transplantable N2.5 tumor model in FVB/N mice, we found that the Treml4-targeted mAb together with Poly ICLC demonstrated significant tumor protection compared with a control non-targeted mAb. Additionally, preliminary ELISAs to determine anti-IgG HER2 levels showed enhanced antibody responses in mice injected with Treml4-Her2 mAb compared with those receiving non-targeted antigen. This suggests an important role of humoral immunity, likely mediated by T-helper cells, in inducing anti-tumor immunity. Future studies will focus on understanding the role of macrophages in anti-tumor immunity. For this, we are engineering HER2/neu to target SIGNR1, a receptor expressed predominantly on splenic marginal zone macrophages, as well as CD11c, which is expressed on a broader range of both DCs and macrophages. We envisage being able to elucidate the role of macrophages and explore the possibility of cross-talk between DCs and macrophages in boosting anti-tumor immunity.

Hotel Floor Plans

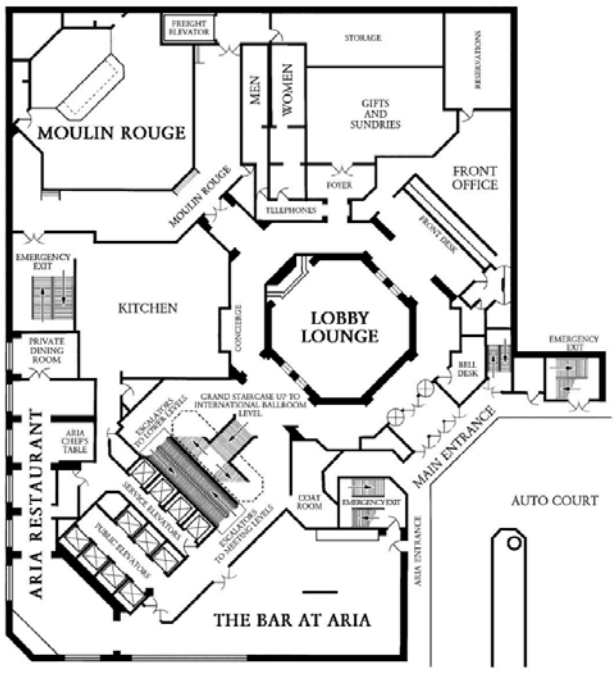
International Ballroom Level (2nd level)



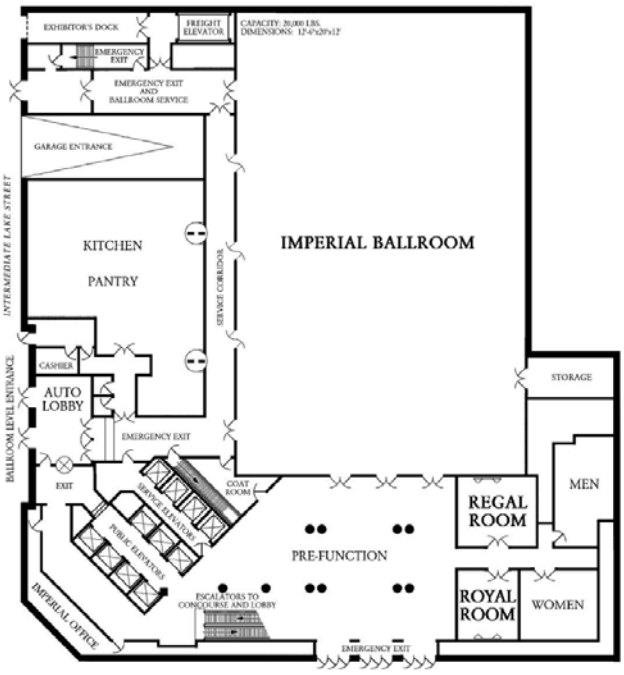
Meeting Room Level (3rd level)



Lobby Level (1st level)



Imperial Ballroom Level (B2)



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