Meeting Program and Abstracts

ASCI / AAP Joint Meeting 2014

April 25 – 27, 2014

The Fairmont Chicago
Chicago, Illinois

American Society for Clinical Investigation 1908

APSA
American Physician Scientists Association

www.jointmeeting.org

Special Events at the 2014 ASCI/AAP Joint Meeting

Friday, April 25

ASCI President's Reception
(by invitation only)

6:15 – 7:15 p.m.
Gold Room (2nd Level)

ASCI Dinner & New Member Induction Ceremony
(ticketed guests only)

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

How to Solve a Scientific Puzzle: Clues from Stockholm and Broadway
Speaker: **Joseph L. Goldstein, MD**
UT Southwestern Medical Center

AAP President's Dinner
(by invitation only)

7:00 – 9:00 p.m.
Mid-America Club (off site)

APSA Welcome Reception & Presidential Address

9:00 p.m. – Midnight
Hancock Center

Speaker: **Evan Noch, MD, PhD**
Weill-Cornell Medical Center

Saturday, April 26

AAP Annual Reception & Dinner

7:00 – 10:00 p.m.
Imperial Foyer & Ballroom (Level B2)

Ending the HIV/AIDS Pandemic: Science and Policy
Speaker: **Anthony S. Fauci, MD**
National Institute of Allergy and Infectious Diseases

APSA Dinner

7:30 – 9:00 p.m.
Moulin Rouge, 1st Floor

The Rotavirus Vaccine: From Bench to Bedside
Speaker: **Paul Offit, MD**
Children's Hospital of Philadelphia

Dessert Reception
(open to all attendees)

10:00 p.m. – Midnight
Imperial Foyer

American Society for Clinical Investigation 1908

APSA American Physician Scientists Association

www.jointmeeting.org

General Information

Accreditation Statement

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 providership 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.

Credit Designation Statement

The University of Chicago Pritzker School of Medicine designates this live activity for a maximum of 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 healthcare professionals will receive a Certificate of Participation. For information on the applicability and acceptance of Certificates of Participation for educational activities certified for *AMA PRA Category 1 Credit™* from organizations accredited by the ACCME, please consult your professional licensing board.

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.

Registration Hours

Friday, April 25	7:00 a.m. – 7:00 p.m.
Saturday, April 26	7:00 a.m. – 5:00 p.m.
Sunday, April 27	7:00 a.m. – 10:00 a.m.

Poster Session Schedule

Friday, April 25

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

Saturday, April 26

Viewing	7:00 a.m. – Noon
Presentation	11:45 a.m. – 1:30 p.m.
Dismantle	1:30 p.m. – 2:00 p.m.

Please be present at your poster during your assigned presentation time.

Best Poster Awards

Best Poster Awards will be given in the amount of $1,000 each. Members of the ASCI, AAP, and APSA will judge posters on scientific novelty, quality and clarity of presentation. **Awards will be presented on Saturday, April 26, at 3:30 p.m.**

Scientific Program

Friday, April 25

Time	Event	Location
7:00 a.m. – 7:00 p.m.	Registration	International Ballroom Foyer
8:30 a.m. – 11:00 a.m.	APSA Business Meeting	Moulin Rouge
11:00 a.m. – 1:00 p.m.	**APSA Session I**	International Ballroom
11:00 a.m. – 11:45 a.m.	**Invited Speaker:** **Mark Courtney, MD** *Northwestern University*	
Noon – 12:45 p.m.	**Invited Speaker:** **Brian Kobilka, MD** *Stanford University*	
1:00 p.m. – 3:00 p.m.	Poster Setup	Imperial Ballroom
1:00 p.m. – 6:00 p.m.	**Plenary Session I:** **Understanding Disease Mechanisms to Improve Human Health** Moderators: **J. Larry Jameson, Mukesh K. Jain, Michael Guo**	International Ballroom
1:00 p.m. – 1:30 p.m.	 **Richard P. Lifton, MD, PhD** *Yale University* **Genomics and the Future of Diagnosis, Prevention and Therapeutics**	
1:30 p.m. – 2:00 p.m.	 **Laurie H. Glimcher, MD** *Weill-Cornell Medical College* **Close to the Bone: Novel Genes that Remodel the Skeleton**	
2:00 p.m. – 3:00 p.m.	**APSA Panel:** **The Intertwining Growth of Women and Medicine in the 21st Century**	Crystal Room
2:00 p.m. – 3:00 p.m.	**ASCI and AAP New Member Presentations**	International Ballroom
2:00 p.m. – 2:15 p.m.	**AAP New Member Presentation:** **John A. Stamatoyannopoulos, MD** *University of Washington School of Medicine* **Decoding the Human Genome**	
2:15 p.m. – 2:30 p.m.	**ASCI New Member Presentation:** **Anthony W. Ferrante, Jr., MD, PhD** *Columbia University College of Physicians & Surgeons* **Immune Regulation of Adipose Tissue Mass**	
2:30 p.m. – 2:45 p.m.	**AAP New Member Presentation:** **Lisa Cooper, MD** *Johns Hopkins University* **The Role of Patient-Physician Relationships in Overcoming Health and Healthcare Disparities**	
2:45 p.m. – 3:00 p.m.	**ASCI New Member Presentation:** **Christoph Klein, MD, PhD** *University Children's Hospital, Dr. von Haunersches Kinderhospital* **Novel Genetic Defects of the Human Immune System**	
3:00 p.m. – 3:30 p.m.	Break	International Ballroom Foyer

Scientific Program

Friday, April 25

Time	Event	Location
3:30 p.m. – 4:00 p.m.	 **José Baselga, MD, PhD** *Memorial Sloan-Kettering Cancer Center* **Targeting the PI3K/AKT/mTOR Pathway in Breast Cancer**	International Ballroom
4:00 p.m. – 4:30 p.m.	 **Carl H. June, MD** *University of Pennsylvania School of Medicine* **Engineering T Cells for Cancer: CARs and Beyond**	International Ballroom
4:30 p.m. – 4:45 p.m.	**JCI Editor Update**	International Ballroom
4:45 p.m. – 5:15 p.m.	 **ASCI Presidential Address:** **Peter Tontonoz, MD, PhD** *University of California, Los Angeles* **Leading by Example: Pastors, Mentors, Physician-Scientists and the ASCI**	International Ballroom
5:15 p.m. – 5:45 p.m.	 **ASCI/Stanley J. Korsmeyer Award Lecture:** **Beth Levine, MD** *UT Southwestern Medical Center* **Autophagy: A Cornerstone of Cellular Health**	International Ballroom
5:45 p.m. – 6:15 p.m.	 **Lasker Award Lecture:** **Napoleone Ferrara, MD** *University of California, San Diego* **Therapeutic Applications of VEGF Inhibitors: Progress and Challenges**	International Ballroom
6:00 p.m. – 9:30 p.m.	Poster Viewing	Imperial Ballroom
6:15 p.m. – 7:15 p.m.	**ASCI Welcome Reception and Poster Viewing**	Imperial Ballroom
7:00 p.m. – 9:00 p.m.	**AAP President's Dinner** (by invitation only)	Mid-America Club
7:00 p.m. – 9:00 p.m.	**APSA Dinner Outing** (on your own) Sign up at the Registration Desk outside the International Ballroom	Off Site
7:30 p.m. – 9:30 p.m.	**ASCI Dinner & New Member Induction Ceremony** (ticketed guests only) Speaker: **Joseph L. Goldstein, MD** *UT Southwestern Medical Center* **How to Solve a Scientific Puzzle: Clues from Stockholm and Broadway**	Moulin Rouge
9:00 p.m. – Midnight	**APSA Welcome Reception & Presidential Address** Speaker: **Evan Noch, MD, PhD** Weill-*Cornell Medical Center*	Hancock Center

Scientific Program

Saturday, April 26

Time	Event	Location
7:00 a.m. – 5:00 p.m.	Registration	International Ballroom Foyer
7:00 a.m. – 8:00 a.m.	Young Investigators' Mentoring Breakfast	Moulin Rouge
7:00 a.m. – 8:00 a.m.	AAP Council Meeting	State Room
8:00 a.m. – 5:45 p.m.	**Plenary Session II:** **Understanding Disease Mechanisms to Improve Human Health** Moderators: **Paul Rothman, Peter Tontonoz, Evan Noch**	International Ballroom
8:15 a.m. – 8:45 a.m.	 **Jean Bennett, MD, PhD** *University of Pennsylvania, School of Medicine* **Gene Therapy for Leber's Congenital Amaurosis as a Stepping Stone for Treating Other Blinding Diseases**	
8:45 a.m. – 9:15 a.m.	 **Martin J. Blaser, MD** *New York University School of Medicine* **Perturbing the Early Life Microbiome and Its Consequences**	
9:15 a.m. – 9:30 a.m.	**APSA Trainee and Oral Abstract Presentation:** **David Y. Chiang** *Baylor College of Medicine* **Ryanodine-Receptor Mediated Calcium Leak Drives Progression of Atrial Fibrillation**	International Ballroom
9:30 a.m. – 10:00 a.m.	 **Christine E. Seidman, MD** *Brigham and Women's Hospital, Harvard Medical School* **Strategies to Fix a Broken Heart**	
10:00 a.m. – 10:30 a.m.	Break	International Foyer
10:30 a.m. – 11:00 a.m.	 **Inaugural ASCI/Harrington Prize Lecture:** **Harry C. Dietz, MD** *Johns Hopkins Medicine* **Found in Translation: New Insights into the Pathogenesis and Treatment of Marfan Syndrome and Related Disorders**	International Ballroom
11:00 a.m. – 11:15 a.m.	**APSA Trainee and Oral Abstract Presentation:** **Jeffrey R. Gehlhausen** *Indiana University School of Medicine* **A Conditional NF2 Mouse Model of Schwannoma Genesis that Recapitulates Human Disease**	International Ballroom
11:15 a.m. – 11:45 a.m.	 **APSA Keynote Speaker:** **Mary Klotman, MD** *Duke University* **From Diagnostics to Theranostics: Expanding Horizons for Clinical Scientists in Imaging**	International Ballroom
11:45 a.m. – 1:30 p.m.	Poster Session with Lunch	Imperial Ballroom and Foyer
12:45 p.m. – 1:30 p.m.	Poster Reviewer Meeting	Royal Room
1:30 p.m. – 2:00 p.m.	Poster Dismantle	Imperial Ballroom

Scientific Program

Saturday, April 26

Time	Event	Location
8:00 a.m. – 5:45 p.m.	**Plenary Session III:** **Understanding Disease Mechanisms to Improve Human Health** Moderators: **Christine E. Seidman, Levi Garraway, Kate Hartmann**	International Ballroom
1:30 p.m. – 2:00 p.m.	 **APSA Keynote Speaker:** **Peter Agre, MD** *Johns Hopkins University* **Opening Doors Worldwide Through Medical Science**	International Ballroom
2:00 p.m. – 3:00 p.m.	**APSA Session II**	International Ballroom
2:00 p.m. – 2:30 p.m.	 **Anthony Atala, MD** *Wake Forest Institute for Regenerative Medicine* **Regenerative Medicine: New Approaches to Healthcare**	
2:30 p.m. – 3:00 p.m.	 **Timothy Ley, MD** *Washington University School of Medicine* **The Acute Myeloid Leukemia Genome(s)**	
2:30 p.m. – 3:00 p.m.	**APSA Global Health Panel**	Crystal Room
2:30 p.m. – 3:00 p.m.	**APSA Public Outreach of the Physician-Scientist**	Gold Room
3:00 p.m. – 3:30 p.m.	Break	International Ballroom Foyer
3:30 p.m. – 3:40 p.m.	**Best Poster Awards**	International Ballroom
3:40 p.m. – 4:00 p.m.	**AAP Business Meeting**	Gold Room
4:00 p.m. – 4:30 p.m.	 **AAP Presidential Address:** **J. Larry Jameson, MD, PhD** *University of Pennsylvania, School of Medicine* **Disruptive Innovation as a Driver of Science and Medicine**	International Ballroom
4:30 p.m. – 5:15 p.m.	**Kober Medal Presentation**  Recipient: **Elizabeth G. Nabel, MD** *Brigham and Women's Hospital, Harvard Medical School*  Presenter: **Eugene Braunwald, MD** *Brigham and Women's Hospital, Harvard Medical School*	International Ballroom
7:00 p.m. – 10:00 p.m.	**AAP Reception and Dinner** Speaker: **Anthony S. Fauci, MD** *National Institute of Allergy and Infectious Diseases* **Ending the HIV/AIDS Pandemic: Science and Policy**	Imperial Ballroom
7:30 p.m. – 9:00 p.m.	**APSA Dinner** Speaker: **Paul Offit, MD** *Children's Hospital of Philadelphia* **The Rotavirus Vaccine: From Bench to Bedside**	Moulin Rouge
10:00 p.m. – Midnight	**Dessert Reception** (open to all attendees)	Imperial Ballroom Foyer

Scientific Program

Sunday, April 27

Time	Event	Location
7:00 a.m. – 10:00 a.m.	Registration	International Ballroom Foyer
8:00 a.m. – 9:30 a.m.	**Interest Groups & Mentorship**	Moulin Rouge
9:00 a.m. – 10:00 a.m.	**ASCI/AAP Joint Council Meeting Wrap-Up**	State Room
9:30 a.m. – 10:00 a.m.	 **APSA Keynote Speaker:** **Hedvig Hricak, MD, PhD** *Memorial Sloan-Kettering Cancer Center* **From Diagnostics to Theranostics: Expanding Horizons for Clinical Scientists in Imaging**	Gold Room
10:00 a.m. – 11:00 a.m.	**APSA Panel Session: Social Sciences and Humanities**	Crystal Room
10:00 a.m. – 11:00 a.m.	**APSA Panel Session: Post-Graduate Opportunities**	Gold Room
11:00 a.m. – Noon	**APSA Panel Session: Policy**	Gold Room
11:00 a.m. – Noon	**APSA Panel Session: MSTP Admissions Panel for Undergraduates**	Crystal Room
Noon – 2:00 p.m.	**APSA Residency Luncheon**	Moulin Rouge

Committee and Faculty

2014 ASCI/AAP Joint Meeting Planning Committee

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

Paul Rothman, MD
AAP President-Elect
Johns Hopkins University School of Medicine

Warner C. Greene, MD, PhD
AAP Immediate Past President
Gladstone Institute of Virology & Immunology

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

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

William C. Hahn, MD, PhD
ASCI Immediate Past President
Harvard Medical School, Dana-Farber Cancer Institute

ASCI Council

Officers

President
Peter Tontonoz, MD, PhD
University of California, Los Angeles, David Geffen School of Medicine

President-Elect
Mukesh K. Jain, MD
Case Western Reserve University School of Medicine

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

Secretary-Treasurer
Judith A. James, MD, PhD
Oklahoma Medical Research Foundation

Editor, *The Journal of Clinical Investigation*
Howard A. Rockman, MD
Duke University School of Medicine

Councilors

Vivian G. Cheung, MD
University of Michigan Medical School

Joel N. Hirschhorn, MD, PhD
Harvard Medical School, Boston Children's Hospital

Anna Huttenlocher, MD
University of Wisconsin

Brendan Lee, MD, PhD
Baylor College of Medicine

Deborah Novack, MD, PhD
Washington University School of Medicine

Norman E. Sharpless, MD
University of North Carolina at Chapel Hill

AAP Council

Officers

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

President-Elect
Paul Rothman, MD
Johns Hopkins University School of Medicine

Secretary
Beverly Mitchell, MD
Stanford Cancer Center

Treasurer
Linda Fried, MD, MPH
Columbia University Mailman School of Public Health

Councilors

John Carethers, MD
University of Michigan

Shaun Coughlin, MD, PhD
Cardiovascular Research Institute, University of California San Francisco

Betty Diamond, MD
Feinstein Institute for Medicine Research, North Shore LIJ Health System

Serpil Erzurum, MD
Lerner Research Institute, The Cleveland Clinic Foundation

Kenneth Kaushansky, MD
Stony Brook University School of Medicine

Mary E. Klotman, MD
Duke University School of Medicine

Stanley Lemon, MD
University of North Carolina at Chapel Hill

Charles Sawyers, MD
Memorial Sloan-Kettering Cancer Center

Christine Seidman, MD
Brigham and Women's Hospital

Stefan Somlo, MD
Yale University School of Medicine

2014 ASCI/AAP Joint Meeting Travel Award Recipients

Ilya O. Blokhin
University of Iowa

Christopher B. Cole
Washington University in St Louis

Ryan B. Day
Washington University in St. Louis

D. Luke Fischer
Michigan State University

Daniel K. Fox
University of Iowa

Jonathan A. Kropski
Vanderbilt University School of Medicine

Alexandra E. Livanos
NYU School of Medicine

Brandon P. Lucke-Wold
WVU School of Medicine

Mei-Sing Ong
University of New South Wales

Jung M. Park
University of Iowa

David A. Russler-Germain
Washington University School of Medicine

Viral S. Shah
University of Iowa

Junko Takeshita
University of Pennsylvania

Glenn T. Werneburg
Stony Brook University

James W. Wisler
Duke University Medical Center

Alexander C. Yang
University of Michigan

Samir Zaidi
Yale University School of Medicine

2014 APSA Annual Meeting Travel Award Recipients

Yohance M. Allette
Indiana University School of Medicine

Emily J. Anstadt
University of Connecticut Health Center

Javier Cabrera-Perez
University of Minnesota

Eileen S. Carpenter
SUNY Stony Brook

Hsiang-Chun Chang
Northwestern University

David Y. Chiang
Baylor College of Medicine

Jeffrey R. Gehlhausen
Indiana University School of Medicine

Kathryn E. Hacker
University of North Carolina at Chapel Hill

Brandon B. Holmes
Washington University in St. Louis

Bianca N. Islam
Georgia Health Sciences University

Cymon N. Kersch
Oregon Health and Science University

Priya Pal
Washington University in St. Louis

James J. Park
Loyola University Chicago

Jennifer Rha
Emory University

Jackeline J. Rodriguez-Smith
National Institute of Arthritis and Musculoskeletal and Skin Diseases

Matthew M. Sheehan
Boston Children's Hospital / Harvard Medical School

Kavisha Singh
Duke University

Emily A. Slat
Washington University in St. Louis

Jennifer A. Stanley
University of Alabama at Birmingham

Daniel Talmasov
Stony Brook University

Nicholas A. Taylor
University of North Carolina at Chapel Hill

Robert L. Trevino
Rush University Medical Center

Maria C. Trissal
Washington University in St. Louis

Cesar A. Virgen
University of California, San Diego

Alice N. Weaver
University of Alabama Birmingham

Reid Wilkening
University of Illinois at Chicago

Jaime E. Zelaya
Oregon Health and Science University

2014 ASCI Council Young Physician-Scientist Awards

The ASCI Council is pleased to recognize the recipients of its 2014 Young Physician-Scientist Awards, which highlight the achievements of early-career investigators. Please visit the ASCI Council Young Physician-Scientist Awards section at the meeting's Poster Session (Saturday, April 26, 11:45 a.m. – 1:30 p.m.) to find out more about their work.

Jason Andrews, MD (Poster: ASCI-1)
Stanford University School of Medicine

Justin R. Bailey, MD, PhD (Poster: ASCI-2)
Johns Hopkins University School of Medicine

David Barbie, MD (Poster: ASCI-3)
Dana-Farber Cancer Institute

Sami Barmada, MD, PhD (Poster: ASCI-4)
University of Michigan

Daniel E. Bauer, MD, PhD (Poster: ASCI-5)
Boston Children's Hospital

Trevor Burt, MD (Poster: ASCI-6)
University of California, San Francisco

Ping Chi, MD, PhD (Poster: ASCI-7)
Memorial Sloan-Kettering Cancer Center

Matthew M. Churpek, MD, MPH, PhD (Poster: ASCI-8)
University of Chicago

Ajai Dandekar, MD, PhD (Poster: ASCI-9)
University of Washington

Andrew Dauber, MD, MMSc (Poster: ASCI-10)
Boston Children's Hospital

Marco L. Davila, MD, PhD (Poster: ASCI-11)
Vanderbilt University

Stephanie Eisenbarth, MD, PhD (Poster: ASCI-12)
Yale University School of Medicine

Joshua A. Englert, MD (Poster: ASCI-13)
Brigham and Women's Hospital/Harvard Medical School

Jorge L. Gamboa, MD, PhD (Poster: ASCI-14)
Vanderbilt University School of Medicine

Don Gibbons, MD, PhD (Poster: ASCI-15)
University of Texas, MD Anderson Cancer Center

Anna Greka, MD, PhD (Poster: ASCI-16)
Harvard Medical School

Alan Hanash, MD, PhD (Poster: ASCI-17)
Memorial Sloan Kettering Cancer Center

Mark Hatley, MD, PhD (Poster: ASCI-18)
St. Jude Children's Research Hospital

Mohit Jain, MD, PhD (Poster: ASCI-19)
University of California, San Diego

Brian S. Kim, MD (Poster: ASCI-20)
Perelman School of Medicine at the University of Pennsylvania

Conor Liston, MD, PhD (Poster: ASCI-21)
Weill Cornell Medical College

Randy Longman, MD, PhD (Poster: ASCI-22)
Weill Cornell Medical College

Ann Mullally, MD (Poster: ASCI-23)
Dana-Farber Cancer Institute

Eirini Papapetrou, MD, PhD (Poster: ASCI-24)
University of Washington

Sudarshan Rajagopal, MD, PhD (Poster: ASCI-25)
Duke University Medical Center

Stacey Rentschler, MD, PhD (Poster: ASCI-26)
Washington University School of Medicine

Andrew Rhim, MD (Poster: ASCI-27)
University of Michigan

Matthew Riese, MD, PhD (Poster: ASCI-28)
Medical College of Wisconsin

Chetan Seshadri, MD (Poster: ASCI-29)
University of Washington

Anthony Shum, MD (Poster: ASCI-30)
University of California, San Francisco

Emily K. Sims, MD (Poster: ASCI-31)
Indiana University School of Medicine

Scott Soleimanpour, MD (Poster: ASCI-32)
University of Michigan Medical School

Stephanie B. Troy, MD (Poster: ASCI-33)
Eastern Virginia Medical School

Young Physician-Scientist Award Abstracts

ASCI-1

Wealth-assortative Social Mixing and the Epidemiological Advantage of Targeting Tuberculosis Interventions to Those Living Below the Poverty Line in India

***Jason Andrews*[1], Sanjay Basu[2], David W. Dowdy[3], and Megan B. Murray[4]**

[1]Division of Infectious Diseases and Geographic Medicine and [2]Stanford Prevention Research Center, Stanford School of Medicine, Stanford, CA; [3]Division of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; [4]Division of Global Health Equity, Brigham and Women's Hospital, Boston, MA

Background: Tuberculosis remains disproportionately concentrated among the poor; however, known risk factors for tuberculosis progression cannot fully explain observed disparities in the burden of tuberculosis. We hypothesized that wealth-assortative mixing, wherein individuals are more likely to contact others from similar socioeconomic backgrounds, may amplify disparities in tuberculosis. **Methods:** We analyzed data from the National Family Health Survey in India to construct composite prevalence odds ratios for tuberculosis according to wealth status, using literature estimates for the magnitude of risk ratios for key risk factors (low BMI, alcohol use, indoor air pollution, diabetes, and tobacco use). We then developed a wealth-structured, mathematical model of tuberculosis transmission to evaluate the potential contribution of differences in time to diagnosis, contact rates, and social mixing patterns. We compared the projected impact of targeted versus untargeted enhanced diagnostic strategies on tuberculosis incidence over ten years. **Results:** Self-reported tuberculosis prevalence among the 40% of the population in India living below the poverty line was 917 cases per 100,000, compared with 354 cases per 100,000 among the wealthiest 60% (prevalence ratio, 2.59). The prevalence ratio projected from known risk factors was 1.40, indicating that these risk factors fail to explain much of tuberculosis disparities. However, if wealth-assortative mixing were present, very small disparities in diagnostic delays, progression rates, or contact rates could generate the large observed disparities. With assortative mixing, we found that an equivalent-scale diagnostic intervention could have a 22% greater impact if targeted to the poorest 40% of the population compared with a population-wide strategy. **Discussion:** Tuberculosis disparities may be amplified by assortative mixing; this factor has not been accounted for in tuberculosis control efforts. In addition to potential efficiencies in targeting higher-risk populations, tuberculosis control efforts would reduce more secondary tuberculosis cases, per primary case diagnosed, if they were preferentially targeted toward the poor.

ASCI-2

Resistance to Multiple Broadly Neutralizing Monoclonal Antibodies is Conferred by Mutations at Non-contact Residues in Hepatitis C Virus E2

***Justin R. Bailey*[1], Lisa N. Wasilewski[1], Anna E. Snider[1], William O. Osburn[1], Steven K.H. Foung[2], and Stuart C. Ray[1]**

[1]Johns Hopkins University School of Medicine, Baltimore, MD; [2]Stanford University School of Medicine, Stanford, CA

Introduction: Isolation of broadly neutralizing monoclonal antibodies (mAbs) against hepatitis C virus (HCV) has raised hope that a vaccine inducing similar neutralizing antibodies (nAbs) could prevent HCV infection. However, mechanisms of resistance to these broadly neutralizing mAbs are poorly understood. **Methods:** We developed a novel panel neutralization method to measure neutralizing breadth of mAbs and identify mutations mediating nAb resistance. A panel of 19 diverse HCV E1E2 (envelope) clones was used to produce a library of HCV pseudoparticles (HCVpp), and we measured neutralization of these HCVpp by 18 published monoclonal anti-HCV broadly neutralizing antibodies (nAbs). E1E2 sequences were analyzed to identify mutations associated with resistance, and the resistance phenotypes of these mutations were confirmed by introduction into nAb-sensitive E1E2 clones. **Results:** We identified envelopes with resistance to each of the 18 previously characterized anti-HCV broadly neutralizing mAbs. Surprisingly, multiple mAbs produced very similar patterns of neutralization across the HCVpp library, and we found significant correlations between rankings of HCVpp resistance to some mAbs thought to have non-overlapping binding sites (e.g., HC84.22 and AR3C, correlation 0.84, $P < 0.0001$). Through sequence analysis of resistant E1E2 clones, we identified a combination of three mutations, I538V/Q546L/T563V, that could confer resistance to at least six broadly neutralizing mAbs, including CBH-2, CBH-5, AR3A, AR3B, AR3C, and HC84.22. **Conclusions:** We have developed a novel method to identify naturally occurring polymorphisms in E1E2 conferring resistance to nAbs. Resistance mutations I538V/Q546L/T563V are not at known contact or binding residues for any of the mAbs to which they confer resistance, and they fall within the central β-sheet of E2 rather than the exposed front sheet/CD81 binding loop that is the likely binding site for most broadly neutralizing mAbs. nAb resistance mutations at non-contact residues could represent a major mechanism of HCV persistence and a potential challenge for HCV vaccine development.

Young Physician-Scientist Award Abstracts

ASCI-3

Inhibition of KRAS-driven Tumorigenicity by Interruption of an Autocrine Cytokine Circuit

Zehua Zhu[1,3], Amir R. Aref[2], Travis J. Cohoon[1], Thanh U. Barbie[4], Yu Imamura[1], Shenghong Yang[1,3], Susan E. Moody[1], Rhine R. Shen[1], Anna C. Schinzel[1], Zhi Rong Qian[1], Jason T. Godfrey[5], Karolina Maciag[3,6], Edmond M. Chan[1,3], Tran C. Thai[1,3], Pablo Tamayo[3], Whitney Silkworth[3], Mary T. Labowsky[1], Lior Rozhansky[1], Jill P. Mesirov[3], William E. Gillanders[4], Shuji Ogino[1], Nir Hacohen[3,6], Suzanne Gaudet[2], Michael J. Eck[2], Jeffrey A. Engelman[5], Ryan B. Corcoran[5], Kwok-Kin Wong[1], William C. Hahn[1,3], and *David A. Barbie*[1,3]

[1]Department of Medical Oncology and [2]Cancer Biology, Dana-Farber Cancer Institute, Boston, MA; [3]Broad Institute of Harvard and MIT, Cambridge, MA; [4]Department of Surgery, Washington University, St. Louis, MO; [5]MGH Cancer Center, Boston, MA; [6]Center for Immunology, Massachusetts General Hospital, Boston, MA

Oncogenic KRAS engages other pathways besides MEK and PI3K that are critical for cell transformation and tumorigenesis, including RAL-TBK1 signaling, NF-κB/STAT3 activation, and cytokine production. Detailed characterization and molecular targeting of these alternate pathways have remained incomplete. We found that TBK1 and its homolog, IKKε, promote RAS transformation by induction of IL-6 and CCL5 autocrine signaling. In addition, we identified the JAK1/2 inhibitor momelotinib (GS-0387/CYT387) as a TBK1/IKKε inhibitor. Momelotinib inhibits TBK1/IKKε activity with nanomolar potency in vitro and suppresses phosphorylation of the TBK1/IKKε target CYLD in cancer cells. Like the TBK1 inhibitor MRT67307 and in contrast to the selective JAK1/2 inhibitor ruxolitinib, momelotinib inhibits IRF3 phosphorylation and the production of TBK1/IKKε-regulated cytokines in LPS-stimulated macrophages. Momelotinib treatment of oncogenic KRAS-driven lung cancer cell lines or genetically engineered Kras mouse lung cancer models inhibits STAT3 and CYLD phosphorylation, suppresses cytokine expression, and leads to prolonged tumor regression. Momelotinib also induces compensatory ERK activation, which is inhibited by combination with the MEK inhibitor selumetinib, resulting in cooperative impairment of cell viability. Treatment of aggressive murine Kras-p53 (KP)-driven lung cancer with momelotinib/selumetinib combination therapy results in synergistic tumor regression and is well tolerated, although resistance developed after 8 weeks. These data highlight the potential of combined JAK/TBK1/IKKε and MEK inhibition as a novel effective therapeutic strategy for KRAS-driven lung cancer.

ASCI-4

Neuronal Autophagy Induction Enhances TDP43 Turnover and Survival in Rodent and Human Models of ALS

***Sami J. Barmada*[1,2,7], Andrea Serio[4], Arpana Arjun[1], Bilada Bilican[4], Aaron Daub[1], D. Michael Ando[1,5], Andrey Tsvetkov[1], Michael Pleiss[6], Xingli Li[7], Daniel Peisach[7], Christopher Shaw[8], Siddharthan Chandran[4], and Steven Finkbeiner[1,2,3,5,6,9]**

[1]Gladstone Institute of Neurologic Disease, San Francisco, CA; Departments of [2]Neurology and [3]Physiology, University of California, San Francisco Medical Center, San Francisco, CA; [4]Euan MacDonald Centre for Motor Neurone Disease Research and Medical Research Council Centre for Regenerative Medicine, University of Edinburgh, Edinburgh, UK; [5]Biomedical Sciences Graduate Program, University of California, San Francisco, CA; [6]Keck Program in Brain Cell Engineering, Gladstone Institutes, San Francisco, CA; [7]Department of Neurology, University of Michigan, Ann Arbor, MI; [8]Institute of Psychiatry, Medical Research Council Centre for Neurodegeneration Research, King's College London, London, UK; [9]Taube-Koret Center for Neurodegenerative Disease Research, Hellman Family Foundation Alzheimer's Disease Research Program, and Roddenberry Stem Cell Program, San Francisco, CA

Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are clinically distinct neurodegenerative disorders that share a common pathology — cytoplasmic inclusions rich in TDP43. Rare TDP43 mutations cause ALS or FTD, but abnormal TDP43 levels and localization may cause disease even if TDP43 lacks a mutation. Here we show that individual neurons vary in their ability to clear TDP43 and are exquisitely sensitive to TDP43 levels. To measure TDP43 clearance, we developed and validated a single-cell method — optical pulse labeling, or OPL — that overcomes confounding effects of aggregation and toxicity and discovered that pathogenic mutations significantly shorten TDP43 half-life. We also adapted OPL into a powerful assay of autophagic flux and confirmed the utility of this method for measuring changes in autophagic activity in living cells. Through a unique and flexible in silico approach to drug discovery, we identified a novel family of compounds that effectively induce autophagic flux in neurons and verified their activity by conventional biochemical means and OPL. These compounds improved TDP43 clearance and localization and enhanced survival in primary murine neurons and in human neurons and astrocytes derived from induced pluripotent stem cells harboring TDP43 mutations associated with familial ALS. Moreover, we successfully calculated the potential improvement afforded by autophagic induction based upon its effects on levels and localization of TDP43. These findings indicate that the levels and localization of TDP43 critically determine neurotoxicity and show that autophagy induction mitigates neurodegeneration by acting directly on TDP43 clearance. Our results thus have significant implications for autophagy inducers as a potential therapeutic strategy in ALS, FTD, and other conditions marked by the deposition of TDP43.

Young Physician-Scientist Award Abstracts

ASCI-5

An Adult Erythroid Enhancer of *BCL11A* Subject to Common Genetic Variation Determines Fetal Hemoglobin Level

***Daniel E. Bauer*[1,2,4], Sophia C. Kamran[4,5], Samuel Lessard[6], Jian Xu[1,4], Yuko Fujiwara[1], Matthew C. Canver[4], Carrie Lin[1], Abhishek Dass[1], Zhen Shao[1], Elenoe C. Smith[1], Luca Pinello[3], Peter J. Sabo[7], Jeff Vierstra[7], Richard A. Voit[8], Guo-Cheng Yuan[3,9], Matthew H. Porteus[8], John A. Stamatoyannopoulos[7], Guillaume Lettre[6], and Stuart H. Orkin[1,2,4,5]**

[1]Division of Hematology/Oncology, Boston Children's Hospital, Boston, MA; [2]Department of Pediatric Oncology and [3]Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Boston, MA; [4]Harvard Medical School, Boston, MA; [5]Howard Hughes Medical Institute, Boston, MA; [6]Montreal Heart Institute and Université Montréal, Montreal, Quebec, Canada; [7]Department of Genome Sciences, University of Washington, Seattle, WA; [8]Department of Pediatrics, Stanford University School of Medicine, Palo Alto, CA; [9]Harvard School of Public Health, Boston, MA

Genome-wide association studies (GWAS) have ascertained numerous trait-associated common genetic variants localized to regulatory DNA, suggesting that regulatory variation may account for substantial heritability. Common variation at *BCL11A* is estimated to explain ~15% of the trait variance in fetal hemoglobin (HbF) level. Here we use chromatin immunoprecipitation (ChIP), DNase I sensitivity assays, and chromosome conformation capture to evaluate the *BCL11A* locus in primary erythroblasts. Common genetic variation at *BCL11A* associated with HbF level lies in noncoding sequences decorated by an erythroid enhancer chromatin signature. We extensively genotype 1,263 samples from the Collaborative Study of Sickle Cell Disease within three HbF-associated erythroid DNase I hypersensitive sites (DHSs) at *BCL11A*. We pyrosequence heterozygous erythroblasts to assess allele-specific transcription factor binding and gene expression. Fine-mapping this putative regulatory DNA uncovers a motif-disrupting common variant associated with reduced transcription factor binding, modestly diminished BCL11A expression, and elevated HbF. We conduct transgenic analysis by mouse zygotic microinjection. The trait-associated sequences function in vivo as a developmental stage-specific lineage-restricted enhancer. We perform genome editing with transcription activator-like effector (TALE) and clustered regularly interspersed short palindromic repeats (CRISPR)/Cas9 nucleases. Genome editing reveals that the enhancer is required in erythroid but dispensable in B-lymphoid cells for expression of BCL11A. We demonstrate a hierarchy of DHSs within the composite enhancer with both evolutionary conservation and functional divergence. We describe a comprehensive and widely applicable approach, including biochemical, genetic, and functional analyses, to reveal causal variants and critical elements. These results validate the hypothesis that common variation modulates cell type-specific enhancers and reveal that although functional variants themselves may be of modest impact, their harboring elements may be critical for appropriate gene expression. We speculate that the GWAS-marked *BCL11A* enhancer represents an attractive target for therapeutic genome editing for the major β-hemoglobin disorders.

ASCI-6

Human Fetal Immune Tolerance and its Impact on Neonatal Immune Function

Trevor D. Burt

Department of Pediatrics, Division of Neonatology, and the Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, University of California, San Francisco, CA

Newborn infection and preterm birth can result in devastating consequences for patients and families, including death of the affected newborn and long-term morbidities in survivors. In order to address these problems, we must first better understand the nature of the fetal and neonatal immune system. T cells in the developing human fetus have a strong tendency to generate immune tolerance and are crucial for establishing tolerance to self and maternal antigens. Fetal and adult T cells have highly distinctive functional characteristics and arise from unique developmentally restricted hematopoietic lineages. We identified a set of genes that are differentially expressed in fetal and adult T cells, and we are leveraging these gene-expression signatures in order to study human fetal and neonatal immune function. Using transcriptional analysis and other techniques, we have demonstrated that both fetal and adult-like cells can be detected in full-term umbilical cord blood and that there is diversity in the range of admixture within a population. This finding suggests that there may be clinically relevant effects of fetal/adult T cell admixture on newborn immune responses. We have established a birth cohort in order to study how vaccine responses are affected by fetal/adult T cell admixture at birth. This study may lead to novel biomarkers that identify infants with poor vaccine responses and who may therefore be more susceptible to infections. We are also carrying out studies in order to understand the normal pace of immune system maturation in the third trimester of human pregnancy and how this normal process is perturbed in the setting of preterm birth. In addition to these clinical/translational studies, we are deeply interested in understanding the basic mechanism by which fetal T cells generate tolerance, with the ultimate goal of being able to harness these mechanisms in order to treat autoimmune diseases.

ASCI-7

Dual Lineage Inhibition of ETV1 and KIT Disrupts the ETV1-KIT Feed-forward Circuit and Potentiates Imatinib Antitumor Effect in GIST Oncogenesis

Leili Ran[1], Inna Sirota[1], Zhen Cao[1], Devan Murphy[1], Shipra Shukla[1], Ferdinando Rossi[4], John Wongvipat[1], William D. Tap[2], Peter Besmer[4], Cristina R. Antonescu[3], Yu Chen[1,2,5], and *Ping Chi*[1,2,5]

[1]Human Oncology and Pathogenesis Program, [2]Department of Medicine, [3]Department of Pathology, [4]Developmental Biology Program, Memorial Sloan-Kettering Cancer Center, New York, NY. [5]Co-corresponding authors.

Gastrointestinal stromal tumor (GIST) is characterized by activating mutations of the KIT or PDGFRA receptor tyrosine kinases and originates from the interstitial cells of Cajal (ICCs), where KIT regulates its normal development and lineage specification. Despite the initial clinical success of imatinib, the majority of patients with advanced GIST develop imatinib resistance and

Young Physician-Scientist Award Abstracts

die of their disease. The development of novel therapeutics that can improve the efficacy of first-line imatinib therapy and/or overcome imatinib resistance is imperative. We have previously identified ETV1, an ETS family transcription factor, as a lineage-specific survival master regulator for GIST and its precursor ICCs. Mutant KIT cooperates with ETV1 in GIST oncogenesis, in part by stabilizing the ETV1 protein through active MAP kinase signaling. Here, we demonstrate that ETV1 is required for GIST initiation and maintenance in vivo using compound genetically engineered mouse models (GEMMs). We have uncovered that ETV1 enhances KIT expression through direct binding to the KIT enhancers. Hence, ETV1 and mutant KIT form a positive feed-forward circuit and dual lineage dependence in GIST pathogenesis, where the ETV1 protein is stabilized by active KIT signaling and stabilized ETV1 augments KIT expression. Further, we demonstrate that inhibition of neither KIT (by imatinib) nor its downstream MAP kinase signaling (by MEK162, a MEK inhibitor) alone is sufficient to durably destabilize the ETV1 protein. Interestingly, the combined targeting of the dual lineage dependence of KIT by imatinib and ETV1 by MEK162 results in durable inhibition of the ETV1 protein and leads to significantly more growth suppression in vitro and complete tumor regression in vivo than either single agent. Our observations demonstrate that ETV1 is a novel therapeutic target in GIST. Importantly, the dual lineage targeting of ETV1 and KIT by the combination therapy may provide a more effective therapeutic strategy than imatinib alone in GIST clinical management.

ASCI-8

Delayed Intensive Care Unit Transfer is Associated with Increased Mortality in Ward Patients

***Matthew M. Churpek*, Trevor C. Yuen, and Dana P. Edelson**
University of Chicago, Chicago, IL

Rationale: Early intervention improves outcomes in many conditions that are indications for intensive care unit (ICU) transfer. However, the impact of delayed transfer on the mortality of critically ill ward patients is poorly characterized. We investigated the impact of delayed transfer on ICU mortality by using the cardiac arrest risk triage (CART) score, a previously published vital sign-based risk score, as an objective measure of critical illness. **Methods:** All patients admitted to the wards at our hospital between November 2008 and January 2011 were included. CART scores were calculated for all patients, and the cut-off corresponding to a specificity of 95% for ICU transfer was defined as the value denoting critical illness. Time from when a patient first reached this CART score until transfer to the ICU was calculated for each patient. Logistic regression was used to calculate the change in odds of death in the ICU for each one-hour delay in ICU transfer time. **Results:** A total of 54,032 admissions occurred during the study period, including 2,166 ICU transfers. The median time from first critical CART score to ICU transfer was 2.7 hours (n = 403). ICU mortality for these patients was 28%, and transfer was delayed for greater than 6 hours in 39%. Each additional one-hour transfer delay was associated with a 7% increase in the odds of ICU mortality ($P < 0.001$). This resulted in an ICU mortality of 21% in the group transferred within the first 6 hours of first reaching the critical CART score value to 52% in the group transferred 18–24 hours after the critical value. **Conclusions:** Delayed transfer to the ICU is associated with increased ICU mortality. Real-time use of an evidence-based early warning score, such as the CART score, could identify critically ill patients on the wards earlier and potentially decrease preventable in-hospital death.

ASCI-9

Adaptation Through Reduction of the Quorum-sensing Regulon by *Pseudomonas aeruginosa*

***Ajai A. Dandekar*[1,2] and E. Peter Greenberg[2]**
Departments of [1]Medicine and [2]Microbiology, University of Washington, Seattle, WA

The major cystic fibrosis lung pathogen *Pseudomonas aeruginosa* has a quorum-sensing system that controls expression of dozens of genes via the acyl-homoserine lactone-responsive transcription factor LasR. Many quorum-controlled genes code for "public goods," products shared among the population. As a result, quorum sensing can be a cooperative behavior under certain circumstances. This leaves the quorum-sensing cooperative groups susceptible to "social cheating" through mutation of LasR; such cheaters save on the cost of public-goods production and therefore grow more rapidly than their wild-type counterparts. LasR mutants accrue over time in chronically colonized CF lungs, although we do not know if these mutants represent social cheaters. There is also evidence to suggest that CF isolates with a functional LasR have comparatively small quorum-sensing regulons. We asked how quickly and dramatically the group of quorum-controlled genes could be reduced through evolution in a laboratory setting. We grew *P. aeruginosa* under conditions that require quorum sensing to obtain carbon and energy and that restrict the emergence of social cheaters to less than 1% of the total population. We used RNA-seq to identify genes in the quorum regulons of whole populations in our evolution experiment. Populations were grown in the presence or absence of an acyl-homoserine-lactone-degrading enzyme to identify quorum sensing-controlled genes. After 900 generations, the number of genes in the quorum-sensing regulon was reduced by 40% compared with the initial regulon. These data suggest that *P. aeruginosa* can effect a relatively rapid reduction in the size of the quorum-sensing regulon. By inference, our results suggest that numerous selective factors work to preserve the large baseline quorum regulon and that the occurrence of LasR mutants and *P. aeruginosa* isolates with relatively small quorum-sensing regulons may reflect an environment where this pathogen depends on quorum sensing for growth.

Young Physician-Scientist Award Abstracts

ASCI-10

Novel Variant in CDKN1C Associated with Intrauterine Growth Restriction, Short Stature, and Early-adulthood-onset Diabetes

Sarah L. Kerns[1]*, Jaime Guevara-Aguirre[2]*, Shayne F. Andrew[3], Juan Geng[4], Carolina Guevara[5], Marco Guevara-Aguirre[5], Michael Guo[6], Yiping Shen[4], Andres Zurita[7], Ron G. Rosenfeld[3], Harry Ostrer[8], Vivian Hwa[9], and *Andrew Dauber*[10]

*[1]Department of Radiation Oncology, Icahn School of Medicine at Mount Sinai, New York, NY; [2]Universidad San Francisco, Quito, Ecuador; [3]Department of Pediatrics, Oregon Health and Science University, Portland, OR; [4]Department of Laboratory Medicine, Boston Children's Hospital, Boston, MA. [5]Institute of Endocrinology, Metabolism, and Reproduction, Quito, Ecuador; [6]Department of Genetics, Harvard Medical School, Boston, MA; [7]Universidad San Francisco, Quito, Ecuador, [8]Departments of Pathology, Pediatrics and Genetics, Albert Einstein College of Medicine of Yeshiva University, New York, NY; [9]Department of Pediatrics, CDRCP, Oregon Health and Science University, Portland, OR; [10]Division of Endocrinology, Boston Children's Hospital, Boston, MA. *These authors contributed equally.*

The CDKN1C gene encodes a cyclin-dependent kinase inhibitor that negatively regulates cellular proliferation. CDKN1C is a paternally imprinted gene, and mutations in CDKN1C are associated with several growth disorder syndromes. Loss-of-function mutations in CDKN1C result in Beckwith-Wiedemann syndrome, which is characterized by prenatal overgrowth and embryonal tumors. Gain-of-function variants cause IMAGe syndrome, which presents with intrauterine and postnatal growth retardation, adrenal hypoplasia, genitourinary anomalies in males, and metaphyseal dysplasia. Recently, loss of function of CDKN1C has been implicated as a possible mechanism in some cases of neonatal hyperinsulinism due to β cell hyperplasia. We studied a large six-generation family with 18 individuals affected by intrauterine growth retardation, severe short stature, and onset of diabetes in early adulthood. The pedigree followed a paternally imprinted pattern of inheritance. We performed genome-wide linkage analysis of 14 individuals and identified a single genome-wide significant 2.6 Mb linkage region on chromosome 11p15 (LOD 3.4). Exome sequencing of 5 individuals resulted in identification of a single novel nonsynonymous variant in the region: a missense variant (p.R281I) in CDKN1C. Segregation analysis via Sanger sequencing showed that all subjects with the clinical phenotype carried this variant. This missense variant lies within the PCNA-binding domain of CDKN1C, two amino acids downstream of the cluster of mutations found to cause IMAGe syndrome. Upon further phenotyping, our subjects did not demonstrate any evidence of adrenal insufficiency, with normal serum ACTH concentrations, but male subjects were uniformly found to have small testicular volumes. Subjects did not have metaphyseal dysplasia. The average height SDS of affected individuals was –5.01 versus –1.96 for controls. The typical age of onset of diabetes was in the mid-30s and was responsive to lifestyle modification and metformin. This family represents an important extension of the phenotypic spectrum of defects in CDKN1C and highlights the possibility of defects in CDKN1C resulting in a novel form of monogenic diabetes.

ASCI-11

Safe and Effective Re-induction of Complete Remissions in Adults with Relapsed B-ALL Using 19-28z CAR CD19-targeted T Cell Therapy

***Marco L. Davila*[1], Jae H Park[2], Isabelle Riviere[2], Xiuyan Wang[2], Shirley Bartido[2], Renier Brentjens[2], and Michel Sadelain[2]**

[1]Vanderbilt University, Nashville, TN; [2]Memorial Sloan-Kettering Cancer Center, New York, NY

Although most adults diagnosed with B-ALL can be induced into a first complete remission (CR1 rates of approximately 80%–90%), a majority will relapse and develop chemorefractory disease. Novel, non-chemotherapy-based treatments are needed for these patients. We have developed a novel CD19-targeted T cell-based therapy for patients with B cell malignancies. We isolate T cells from patients with relapsed/refractory B-ALL and genetically modify them with a chimeric antigen receptor (CAR) construct, termed 19-28z, which comprises a CD19 binding domain fused to the signaling domains of the CD28 costimulatory receptor and the ζ chain of the CD3 complex. We now describe the results from our phase I protocol infusing 19-28z T cells into adults with relapsed/refractory B-ALL (NCT01044069). Sixteen adults have been treated to date. Eight of the patients were infused with 19-28z CAR T cells while they had gross residual disease (>5% to 70% blasts in the BM). The remaining patients had minimal residual disease (MRD). Seven patients developed toxicities including high-grade fevers (>40°C), hypotension, hypoxia, mental status changes, and seizures. These episodes ran for approximately 1 week before they were halted by treatment with steroids or tocilizumab. The other 9 patients did not experience toxicities, and all patients completely recovered and were able to leave the hospital. The occurrence of toxicities correlated with tumor burden, so that patients with gross residual disease (>5% blasts in BM) developed toxicities, while patients with MRD had no evidence of toxicities. The complete remission (CR) rate seen in all patients was 88%, which is much higher than expected with chemotherapy alone (30%). Furthermore, these were high-quality molecular remissions, as assayed by molecular or flow cytometry assays. Overall, the results from this phase I protocol demonstrate that toxicities associated with this therapy are predictable and manageable. Furthermore, the remarkable rates of reinduction of molecular and complete remissions indicate that this therapy warrants further evaluation in a phase II protocol.

ASCI-12

Dendritic Cell Migration During Vaccination

Jayendra Kumar Krishnaswamy, Arpita Singh, and *Stephanie C. Eisenbarth*

Departments of Laboratory Medicine and Immunobiology, Yale University School of Medicine, New Haven, CT

The primary antigen-presenting cell that primes a naive T cell during vaccination is the dendritic cell (DC). DCs function as sentinels to detect invasion and respond by upregulating requisite T cell priming signals and also by migrating to the site of naive T cell priming, the lymph node (LN). Although this latter step is crucial for a DC to interact with T cells, the molecular regulation of DC exit from sites of immunization is not known. We recently discovered, using a murine system, that one member of the

Young Physician-Scientist Award Abstracts

NOD-like receptor family of innate immune molecules, NLRP10, controls the ability of DCs to reach draining LNs. Accordingly, loss of NLRP10 results in a global defect in adaptive immunity upon immunization with an antigen in multiple adjuvants. Using in vivo tracking techniques, we determined that this loss of adaptive immunity was due to a DC-intrinsic failure to emigrate out of inflamed tissues following antigen capture. Actin polymerization and migration of NLRP10-deficient DCs, which require intact CCR7 signaling, in multiple 2D assays indicated no defect in DC movement. Therefore, we engineered a 3D artificial extracellular matrix that recapitulates inflamed tissue; we can image active DC movement through these matrices toward LN-homing chemokine gradients such as CCL19. Use of these systems uncovered a failure in leading-edge coordination by activated DCs deficient in NLRP10 during directed movement, suggesting defective signaling in pathways that control the small GTPase Cdc42. Indeed, regulation of Cdc42 during particular stages of DC maturation appears to be affected in NLRP10-deficient DCs. We are now testing whether the same regulatory pathway exists in human DCs and ultimately whether alterations in DC migration potentially affect the response to vaccination.

ASCI-13

Zinc Deficiency Primes the Lung for Ventilator-induced Lung Injury

***Joshua A. Englert*[1], Francis Boudreault[2], Miguel Pinilla-Vera[1], Alvin T. Kho[3], Carolina Quintana[1], Diana Amador-Munoz[1], Daniel J. Tschumperlin[2], and Rebecca M. Baron[1]**
[1]Pulmonary and Critical Care Division, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; [2]Molecular and Integrative Physiological Sciences Program, Department of Environmental Health, Harvard School of Public Health, Boston, MA; [3]Children's Hospital Informatics Program, Harvard-MIT Division of Health Sciences and Technology, Boston, MA

Mechanical ventilation is a necessary intervention to support patients with acute lung injury, but it can also exacerbate injury through mechanical stress-activated signaling pathways. We show that stretch applied to isolated human lung cells, and to mouse lungs in vivo, induces robust expression of metallothionein, a potent antioxidant and cytoprotective molecule critical for cellular zinc homeostasis. Genetic deficiency of murine metallothionein genes exacerbates lung injury caused by high tidal volume mechanical ventilation, identifying an adaptive role for these genes in limiting lung injury. Stretch induction of metallothionein requires zinc and the zinc binding transcription factor MTF-1. We show that dietary zinc deficiency in mice potentiates ventilator-induced lung injury and that plasma zinc levels are significantly reduced in human patients who go on to develop acute respiratory distress syndrome (ARDS) compared with healthy and non-ARDS ICU controls. Taken together, our findings identify a novel adaptive response of the lung to stretch and a critical role for zinc in defining the lung's tolerance for mechanical ventilation. These results demonstrate that failure of stretch-adaptive responses play an important role in exacerbating mechanical ventilator-induced lung injury and identify zinc and metallothionein as targets for developing lung-protective interventions in patients requiring mechanical ventilation.

ASCI-14

Increasing Mitochondrial Dysfunction with Severity of Chronic Kidney Disease

***Jorge Gamboa*, F. Tremaine Billings IV, and Nancy J. Brown**
Vanderbilt University School of Medicine, Nashville, TN

Cardiovascular mortality increases as kidney function declines. Markers of oxidative stress predict cardiovascular complications in patients with chronic kidney disease (CKD). Mitochondrial dysfunction leads to oxidative stress and may contribute to the pathogenesis of cardiovascular disease in patients with CKD. We tested the hypothesis that mitochondrial function worsens with severity of CKD by assessing mitochondrial DNA (mtDNA) copy number in peripheral blood mononuclear cells (PBMCs), plasma lactate, and plasma isofurans. Isofurans are formed preferentially over F2-isoprostanes by non-enzymatic oxidation of arachidonic acid when oxygen tissue tension is high, and they have been proposed as markers of mitochondrial dysfunction. We first validated isofurans as biomarkers of mitochondrial toxicity in doxorubicin-treated mice (renal isofurans 7.04 ± 0.68 versus 5.72 ± 0.26 ng/g of tissue in controls). We next studied 201 subjects classified by severity of CKD based on eGFR: stages 1–2 (n = 105), stages 3–4 (n = 51), and stage 5 (hemodialysis, n = 74). Groups were comparable in age, sex, BMI, and prevalence of hypertension. mtDNA copy number was significantly lower in patients with stage 5 CKD (mtDNA/nDNA 1.72, 95% CI 0.10–3.34) than in patients with stages 3–4 CKD (3.44, 95% CI 0.98–5.90, P = 0.02) and stages 1–2 CKD (6.56, 95% CI 4.57–8.54, P = 0.008). Plasma lactate levels were higher in patients with stage 5 CKD (median 0.61 nM, IQR 0.27–1.08) than in patients with stages 3–4 CKD (median 0.19 nM, IQR 0.07–0.57, P = 0.004) and stages 1–2 CKD (median 0.18 nM, IQR 0.1–0.55, $P < 0.001$). Isofurans were also higher in patients with stage 5 CKD (median 59.72 pg/ml, IQR 43.13–91.03) compared with stages 3–4 CKD (median 48.41 pg/ml, IQR 30.93–66.19, P = 0.01) and stages 1–2 CKD (median 39.00 pg/ml, IQR 26.22–67.03, P = 0.005), whereas F2-isoprostanes did not differ among CKD groups. Progressive kidney disease is associated with worsening mitochondrial dysfunction. Further studies are required to evaluate in vivo mitochondrial function in patients with different CKD stages.

ASCI-15

miR-200 Controls Matrix Deposition and Matrix-Dependent Src Signaling to Control NSCLC Invasion and Metastasis

***Don L. Gibbons*[1,2], Christin Ungewiss[1], Young-Ho Ahn[1], Yanan Yang[1], Lauren A. Byers[1], Mark Schliekelman[3], Zain H. Rizvi[1], Chad J. Creighton[4], and Jonathan M. Kurie[1]**
[1]Department of Thoracic/Head and Neck Medical Oncology and [2]Department of Molecular and Cellular Oncology, MD Anderson Cancer Center, Houston, TX; [3]Fred Hutchinson Cancer Research Center, Seattle, WA; [4]Dan L. Duncan Cancer Center, Baylor College of Medicine, Houston, TX

Non-small cell lung cancer (NSCLC) is paradigmatic of metastatic epithelial tumors with poor treatment options and is the leading cause of cancer-related death, accounting for 160,000 deaths annually in the United States. Normal epithelial integrity is abrogated during the metastatic process, with marked alterations

in cell-cell and cell-extracellular matrix (ECM) interactions that maintain proper epithelial architecture. Using a robust autochthonous genetic murine model (GEMM) harboring the two most common genetic aberrations found in human lung adenocarcinoma (mutant Kras and p53), we have shown that loss of microRNA-200 family (miR-200) expression drives an epithelial-mesenchymal transition (EMT), with a fulminant metastatic phenotype. Using this model and cancer cell lines derived from it, we have demonstrated that miR-200 loss is necessary and sufficient to activate the EMT program, driving tumor invasion and metastasis. We confirmed these results in human lung cancer cell lines and patient samples from The Cancer Genome Atlas lung adenocarcinoma project. Interestingly, miR-200 repression occurs in a subset of tumor cells at the tumor-stromal interface, regulating the tumor microenvironment by altering ECM composition and enhancing tumor cell-matrix interactions that drive secondary tumor cell activation. miR-200 normally represses the expression of multiple ECM components that produce a stiff, fibrotic matrix. In concert, miR-200 acts to regulate ECM-mediated cellular activation by dampening signaling through the integrin β1/Fak/Src pathway. Biochemical analyses of murine and human NSCLC cell lines with differential miR-200 expression demonstrated broad signaling pathway dysregulation with miR-200 loss, including Src/Fak/Pax phosphorylation that was dependent upon collagen I-integrin β1 interactions. Pharmacologic or RNAi-mediated inhibition of this axis significantly reduced migration/invasion in 2D and 3D culture conditions. Our findings suggest that tumor cell microRNA changes produce a cell-intrinsic EMT and complementary changes in the surrounding microenvironment that enhance tumor cell-matrix interactions, driving tumor cell activation and invasive/metastatic potential.

ASCI-16

Inhibition of the TRPC5 Ion Channel is a Novel Therapeutic Strategy for Glomerular Kidney Disease

Thomas Schaldecker[1], Sookyung Kim[1], Constantine Tarabanis[1], Dequan Tian[1], Philip Castonguay[1], Corey R. Hopkins[2], Craig W. Lindsley[2], Lisa Buvall[1], Astrid Weins[1,3], and *Anna Greka*[1]

[1]Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA; [2]Departments of Chemistry and Pharmacology, Vanderbilt Center for Neuroscience Drug Discovery, MLPCN Specialized Chemistry Center, Institute for Chemical Biology, Vanderbilt University Medical Center, Nashville, TN; [3]Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

Ion channels are central to virtually all aspects of cell behavior, and yet their role in the regulation of kidney filter function remains obscure. The human kidney filter handles 180 liters of plasma each day, sorting molecules for passage into the urine or retention in the blood stream. Upon filter barrier damage, plasma albumin spills into the urinary space, a pathologic condition known as albuminuria. Albuminuria is an early marker of progressive kidney disease and, more important, an independent predictor of metabolic syndrome and cardiovascular disease such as heart failure and stroke. Due to the growing epidemic of metabolic syndrome, there is now an urgent unmet need for targeted therapies to prevent albuminuria. Specialized kidney pericytes, known as podocytes, rely on dynamic actin remodeling to safeguard glomerular filter function. Thus, podocyte injury and death are central to the emergence of albuminuria. Transient receptor potential classical (TRPC) channels are calcium-permeable cationic channels with diverse functions. We have previously shown that TRPC5 channels activate the small Rho protein Rac1 to mediate maladaptive cytoskeletal remodeling in podocytes. In this study, we show that the ion channel TRPC5 is a critical mediator of filter damage. Using a recently discovered small molecule inhibitor of TRPC5, patch clamp electrophysiology, and a novel calcium imaging technique in isolated kidney glomeruli, we show efficient podocyte TRPC5 inhibition ex vivo as well as in vitro. Using real-time imaging of the podocyte actin cytoskeleton, we further show that podocyte TRPC5 inhibition abrogates cytoskeletal collapse in vitro, by blocking the activity of Rac1 and by stabilizing the podocyte-specific cytoskeletal modulator synaptopodin. TRPC5 inhibition is also shown to protect from calcium-mediated mitochondrial toxicity and prevent podocyte death. Importantly, genetic deletion or pharmacologic inhibition of TRPC5 protects mice from filter damage in two acute models of disease. Furthermore, we show that TRPC5 inhibition in a rat model of progressive kidney disease ameliorates proteinuria and protects from kidney disease progression. This study identifies TRPC5 inhibition as a therapeutic strategy for the prevention or treatment of albuminuria and progressive kidney disease.

ASCI-17

IL-22 Promotes Intestinal Stem Cell Proliferation and Recovery from Immune-mediated Damage

Caroline A. Lindemans, Anna Mertelsmann, Jarrod A. Dudakov, Enrico Velardi, Guoqiang Hua, Margaret O'Connor, Richard Kolesnick, Marcel R.M. van den Brink, and *Alan M. Hanash*

Memorial Sloan Kettering Cancer Center, New York, NY

IL-22 maintains mucosal barrier function after intestinal injury, but the cellular targets of IL-22 leading to epithelial recovery are poorly understood. To test the effects of IL-22 on intestinal regeneration, we utilized a graft-versus-host disease (GVHD) model of immune-mediated gut damage. Daily i.p. administration of recombinant murine IL-22 starting one week after LP→C57BL/6 (H-2b→H-2b) bone marrow transplantation led to decreased pathology in small and large intestine three weeks after transplant ($P < 0.001$). IL-22 administration had no effect on gut lymphocyte infiltration, inflammatory cytokine production, or flora composition. Investigating the direct effects of IL-22 on the epithelial compartment of the small intestine, we found that IL-22 administration increased proliferation of $Lgr5^+$ intestinal stem cells (ISCs) as measured by Ki-67 expression and mitigated the loss of ISCs in GVHD ($P < 0.05$). In contrast, IL-22 administration had no impact on GVHD-related loss of Paneth cells or on small intestine Wnt3 and EGF mRNA, suggesting a direct effect on ISCs, not a manipulation of their niche. In summary, we found that IL-22 is able to influence epithelial regeneration in vivo, promoting ISC proliferation and recovery from immune-mediated damage.

Young Physician-Scientist Award Abstracts

ASCI-18

A Mouse Model of Rhabdomyosarcoma Originating from a Non-muscle Lineage

***Mark E. Hatley*[1], Matthew R. Garcia[1], David Finkelstein[2], Wei Tang[3], Jonathan Graff[3], Rene L. Galindo[4,5], and Eric N. Olson[5]**

[1]Department of Oncology and [2]Department of Biostatistics, St. Jude Children's Research Hospital, Memphis, TN; [3]Department of Developmental Biology, [4]Department of Pathology, and [5]Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in children. Despite aggressive chemotherapy, radiotherapy, and surgery, clinical outcomes for RMS have not improved for three decades, emphasizing the need to uncover the molecular underpinnings of the disease. RMS is an aggressive skeletal muscle lineage tumor composed of malignant myoblasts that fail to exit the cell cycle and are blocked from fusing into syncytial muscle. RMS includes two histolopathologic subtypes: alveolar RMS, driven by the fusion protein PAX3/7-FOXO1, and embryonal RMS (ERMS), which are genetically heterogeneous. Here, we show that activation of sonic hedgehog signaling through expression of a constitutively active Smoothened allele, SmoM2, under control of an aP2 adipocyte-restricted transgene, aP2-Cre, in mice gives rise to aggressive skeletal muscle tumors that display the histologic and molecular characteristics of human ERMS. In this model, tumorigenesis occurs with high penetrance (~80%), is early onset (by 2 months of age), and is restricted to the head and neck. Also, unlike previous RMS models, this model requires no additional background mutations, such as inactivation of p53, and drives only ERMS neoplasia. We illustrate that the transcriptome of the aP2-Cre;SmoM2 tumors recapitulates both other mouse ERMS models and human ERMS. We also illustrate that the aP2-Cre transgene is not expressed in the skeletal muscle and the activation of the SmoM2 allele directly in muscle satellite cells does not result in tumor formation. Our findings suggest that adipocyte progenitors are a potential cell of origin for sonic hedgehog-driven ERMS, showing that RMS can originate from non-skeletal muscle precursors. We postulate that the relatively short latency and high penetrance of ERMS in this model now provides an efficient genetic system and tool for the analysis of RMS modifiers and provides a unique platform for preclinical studies.

ASCI-19

Systematic Identification of Metabolic Vulnerabilities in Cancer

***Mohit Jain*[1,2,3,4], Roland Nilsson[1,2,3,5], Nikhil Madhusudhan[1,2,3], Laura Strittmatter[1,2,3], Clary B. Clish[1], and Vamsi K. Mootha[1,2,3]**

[1]Broad Institute, Cambridge, MA; [2]Harvard Medical School, Boston, MA; [3]Massachusetts General Hospital, Boston, MA; [4]University of California, San Diego, La Jolla, CA; [5]Karolinska Institute, Stockholm, Sweden

Metabolic reprogramming is an established hallmark of cancer, yet the full complement of metabolic alterations that occur with cancer transformation has yet to be characterized, and the role of metabolic reprogramming in promoting cancer proliferation and survival remains unclear. Using mass spectrometry-based metabolomics, we have quantified the consumption and release of 219 metabolites from media across a panel of 60 diverse human cancer cell lines. These analyses reveal that consumption of extracellular glycine is strongly related to proliferation rate across cancer cells but not primary, non-transformed human cells. Consumed glycine was found to selectively contribute to de novo purine biosynthesis, and antagonizing glycine uptake and its biosynthesis selectively impaired rapidly proliferating cancer cells. To identify cancer-specific metabolic enzymes whose expression may underlie metabolic dysregulation in cancer, we subsequently investigated mRNA profiles for 1,454 metabolic enzymes across 1,981 diverse tumors and normal tissue counterparts. This meta-analysis found the mitochondrial glycine biosynthesis pathway to be highly overexpressed in cancer, and in particular the enzyme methylenetetrahydrofolate dehydrogenase (MTHFD2). MTHFD2 expression was abundant in embryonic development and silenced in adult tissues with counter-expression of its cytosolic paralog, MTHFD1. Moreover, expression of MTHFD2 was highly predictive of survival in multiple independent cohorts of patients with breast cancer. Genetic silencing of MTHFD2 selectively inhibited cancer cell proliferation and resulted in widespread cellular death, establishing MTHFD2 as a unique cancer-specific metabolic activity that may in principle be targeted for cancer therapy. Through large-scale chemical screening, we have now identified initial inhibitors of MTHFD2. This work collectively highlights the use of mass spectrometry-based metabolomics, genomics, integrative analysis, and metabolic biochemistry to systematically chart the metabolic dysregulation that underlies cancer transformation, as well as subsequent identification of glycine metabolism and the cancer-specific enzyme MTHD2 as unique metabolic vulnerabilities that may be targeted for cancer therapeutics.

Young Physician-Scientist Award Abstracts

ASCI-20

Innate Lymphoid Cells Promote Skin Inflammation

***Brian S. Kim*[1,2,3], Mark C. Siracusa[1,2], Kelvin Wang[1,2,3], Steven A. Saenz[1,2], Masato Kubo[5,6], and David Artis[1,2,4]**

[1]Department of Microbiology, [2]Institute for Immunology, and [3]Department of Dermatology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; [4]Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA; [5]Laboratory for Cytokine Regulation, Research Center for Integrative Medical Science, RIKEN Yokohama Institute, Kanagawa, Japan; [6]Division of Molecular Pathology, Research Institute for Biomedical Science, Tokyo University of Science, Chiba, Japan

Innate lymphoid cells (ILCs) are a recently identified family of immune cells that regulate immunity, inflammation, and tissue repair at epithelial barrier surfaces. Previous studies have demonstrated that group 2 ILCs (ILC2s) found in the intestine or lung respond to the epithelial cell-derived cytokines IL-25 and IL-33. We identified that ILC2s accumulate in the skin lesions of atopic dermatitis (AD) patients. In addition, ILC2s accumulated in lesional AD-like skin in a murine model of AD and contributed to the development of cutaneous inflammation. Remarkably, ILC2 responses and AD-like inflammation were independent of IL-25 and IL-33, but critically dependent on the epithelial cell-derived ctyokine thymic stromal lymphopoietin (TSLP). However, the cellular mechanisms through which ILC2s promote cutaneous inflammation remain poorly characterized. Employing immunofluorescence microscopy, we are examining the anatomical distribution of ILC2s in healthy and inflamed skin and testing their capacity to regulate other innate and adaptive immune cell responses to orchestrate the cascade of events that promote cutaneous inflammation.

ASCI-21

Development of a Diagnostic and Prognostic Neuroimaging Biomarker of Major Depressive Disorder

Andrew T. Drysdale, Benjamin D. Zebley, Marc J. Dubin, and *Conor Liston*

Brain and Mind Research Institute and Department of Psychiatry, Weill Cornell Medical College, New York, NY

Translational and clinical studies of major depressive disorder (MDD) are hindered by the lack of an objective biomarker linked to a specific pathophysiology and capable of quantifying or predicting treatment outcomes. Subgenual cingulate hyperactivity has emerged as a consistent abnormality and important therapeutic target in MDD and may relate to deficits in cortical GABA availability. Using optogenetic tools to recapitulate this pathology in rats, we found that enhancing medial prefrontal (mPFC) excitability triggers anhedonic behavior and increases fMRI measures of connectivity with limbic brain structures implicated in reward processing. Similarly, in patients with MDD, we identified analogous abnormalities in GABA availability and mPFC connectivity that were predictive of treatment responsiveness. However, high intersubject variability in these measures limits their clinical utility and may reflect a syndrome that encompasses multiple heterogeneous but clinically indistinguishable neuropathologies. If so, then the key to biomarker development may lie in assembling large datasets to empirically identify distinct neural correlates of depression in homogeneous subgroups of patients. We tested this hypothesis using resting-state fMRI scans in 747 patients with MDD and matched controls. We characterized three relatively homogeneous subtypes of MDD with distinct patterns of abnormal corticocortical connectivity. These potential biomarkers could reliably identify actively depressed patients and likely treatment responders, with sensitivity and specificity exceeding 90% in a training dataset and comparable rates in cross-validation and treatment prediction analyses. These results underscore the potential for functional connectivity measures to serve as diagnostic and prognostic biomarkers and inform clinical decision making.

ASCI-22

Microbiota Regulate CX_3CR1^+ Mononuclear Phagocytes to Support Colitis-associated Innate Lymphoid Cell Production of IL-22

***Randy S. Longman*[1,2], Gretchen E. Diehl[1], Daniel Victorio[1,2], Jun R. Huh[1], Carolina Galan[1], Arun Swaminath[3], Emily Miraldi[1], Richard Bonneau[1], Ellen Scherl[2], and Dan R. Littman[1,4]**

[1]New York University School of Medicine, New York, NY; [2]The Jill Roberts Center for IBD, Weill Cornell Medical College, New York, NY; [3]Columbia University Medical Center, New York, NY; [4]Howard Hughes Medical Institute

Interleukin-22 (IL-22)-producing innate lymphoid cells (ILC3) promote mucosal healing and maintain barrier integrity. The role of these cells in inflammatory bowel disease (IBD) and their functional regulation by intestinal microbiota have not been elucidated. Here, we show that ILCs from the colonic lamina propria of patients with mild to moderate ulcerative colitis and colonic Crohn's disease have increased IL-22 production. Notably, the abundance of colitis-associated IL-22 production by ILC3 correlates with exposure to luminal microbiota in both humans and mouse models. Toll-like receptor (TLR)-MyD88-dependent production of IL-23 and IL-1β by $CD11c^+$ cells was required to support colitis-associated ILC3 production of IL-22 and promote mucosal healing. Both human and mouse CX_3CR1^+ lamina propria mononuclear phagocytes (MNPs) were more efficient than conventional $CD103^+$ dendritic cells in supporting IL-22 production by ILC3 in vitro. Selective depletion of CX_3CR1^+ MNPs using cell-specific expression of the diphtheria toxin receptor revealed the importance of these cells in vivo for ILC3-dependent protection from *Citrobacter rodentium*-mediated colitis. These results reveal the importance of CX_3CR1^+ MNPs in integrating microbial signals to regulate ILC3 function in IBD.

Young Physician-Scientist Award Abstracts

ASCI-23

Distinct Effects of Tet2 Loss and Jak2V617F Expression in Different Hematopoietic Compartments Combine to Promote Disease Progression in Myeloprolfierative Neoplasms (MPN)

Edwin Chen[1], Lawrence J. Breyfogle[1], Rebekka K. Schneider[1], Luke Poveromo[1], Kristina Brumme[1], Ross Levine[3], Benjamin L. Ebert[1,2], and *Ann Mullally*[1,2]

[1]Division of Hematology, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; [2]Broad Institute, Cambridge, MA; [3]Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, NY

Mutations in genes involved in epigenetic regulation (e.g. *TET2*) frequently co-occur with signaling mutations (e.g., *JAK2V617F*) in myeloproliferative neoplasms (MPN). Clinical correlative studies have demonstrated that epigenetic mutations are enriched in more advanced phases of MPN such as myelofibrosis, suggesting that alterations in epigenetic genes cooperate with signaling mutations to drive disease progression in MPN. While clonal analysis of patient samples and studies in immunodeficient mice have provided some insights into *TET2-JAK2V617F* co-mutation in MPN, a detailed dissection of the combined functional effects of these genetic alterations within distinct hematopoietic compartments in vivo is currently lacking. Using genetic murine models, we investigated the impact of *Tet2* loss on *Jak2V617F*-mediated MPN. We found that in *Jak2V617F* mice, *Tet2* loss promoted hematopoietic stem cell (HSC) expansion in the spleen and enhanced extramedullary hematopoiesis and splenomegaly. We found that *Tet2* loss conferred a functional competitive advantage to *Jak2V617F*-mutant HSCs and, concordant with this, that *Jak2V617F* expression and *Tet2* loss activated distinct transcriptional programs in the HSC compartment. In aggregate, our findings indicate that *Tet2* loss drives clonal dominance in HSCs and *Jak2V617F* expression causes expansion of downstream precursor cell populations, resulting in disease progression through combinatorial effects in *Jak2V617F/Tet2* compound mutant mice.

ASCI-24

Haploinsufficient Gene Discovery with Novel hPSC-based Models of Myelodysplasia

Andriana G. Kotini, Ibrahim Boussaad, and *Eirini P. Papapetrou*

Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY

With recent advances in genomic technologies, genetic determinants of human disease are being catalogued at an unprecedented rate, but functional tools to mine them are lagging behind. The most powerful approach to attribute causality and assign function to disease-associated genetic variants is through experimental ablation or introduction in an isogenic setting. However, many genetic lesions, particularly large lesions, are intractable with existing tools, because of a need for a physiologic genomic and cellular context. We used cellular reprogramming and genome engineering to, respectively, "capture" and "reproduce" deletions of chromosome 7q — strongly associated with myelodysplastic syndromes (MDS) and other myeloid malignancies — in isogenic human pluripotent stem cells (hPSCs). We characterized cellular phenotypes relevant to myelodysplasia (reduced hematopoietic differentiation potential and clonogenic capacity and excessive cell death) and show that they are rescued by spontaneous duplication and recapitulated by engineered hemizygosity of defined chromosome 7q regions, overlapping in a 20 Mb fragment spanning bands q32.3–q36.1, providing the first definitive evidence that the underlying mechanism is reduced gene dosage (haploinsufficiency). To identify the critical gene(s) on chromosome 7q, we selected 62 candidate haploinsufficient genes (with significantly reduced expression in del[7q]- compared with isogenic normal iPSCs), constructed a barcoded lentiviral library, and performed a pooled library screen for genes that rescue hematopoiesis in del(7q)-MDS-iPSCs. The top hit was EZH2, a gene known to recurrently harbor loss-of-function mutations in MDS, validating this strategy. This and three additional hits are currently being further validated. In conclusion, we used for the first time hPSC platforms for functional mapping of a critical chromosomal region and discovery of haploinsufficient tumor suppressor genes. Our study presents a powerful and timely approach for elucidating the genetic mechanisms of human disease and interpreting genetic variation.

ASCI-25

Beta-arrestin 1 Regulates BMPR-II Signaling and the Development of Pulmonary Arterial Hypertension

***Sudarshan Rajagopal*[1], Jeffrey J. Kovacs[1], Cristian T. Badea[2], Brian L. Brockway[1], Joshua C. Snyder[1], Darin P. Clark[2], Barry R. Stripp[3], Howard A. Rockman[1], and Claude A. Piantadosi[1]**

[1]Department of Medicine and [2]Department of Radiology, Duke University Medical Center, Durham, NC; [3]Department of Medicine, Cedars Sinai Medical Center, Los Angeles, CA

Pulmonary arterial hypertension (PAH) is a disease associated with elevated pulmonary vascular resistance that leads to right ventricular (RV) hypertrophy, dilation, and ultimately failure. PAH is often associated with changes in signaling by transmembrane receptors, most commonly with decreased signaling by the type II bone morphogenetic protein receptor (BMPR-II), a canonical, type II TGF-β receptor. BMPR-II signals are transduced through the phosphorylation of downstream effector Smads, and gene mutations that decrease BMPR-II activity are thought to increase susceptibility to PAH. In this work, we find that β-arrestin 1 (βarr1), a versatile adapter protein known to regulate signaling by seven-transmembrane receptors (7TMRs), binds to and regulates signaling by BMPR-II. Downstream of BMPR-II activation, siRNA-mediated knockdown of βarr1 decreases Smad phosphorylation and decreases expression of a reporter downstream of a BMP-responsive element. Mirroring these findings at the biochemical and transcriptional level, βarr1-KO mice develop significantly worse hypoxia-induced pulmonary hypertension and RV dysfunction compared with WT mice. These results demonstrate that βarr1 regulates BMPR-II signaling and the development of PAH, suggesting that increasing βarr-mediated signaling may be of therapeutic utility in PAH.

Young Physician-Scientist Award Abstracts

ASCI-26

Canonical Wnt Signaling Regulates Atrioventricular Junction Maturation and is Dysregulated in a Murine Model of Ventricular Preexcitation

Benjamin Gillers[1]*, Haytham Aly[1]*, Aditi Chiplunkar[1], Tomas Valenta[2], Konrad Basler[2], and *Stacey Rentschler*[1]

[1]Washington University School of Medicine, Department of Medicine and Department of Developmental Biology, St. Louis, MO; [2]Institute of Molecular Life Sciences, University of Zurich, Zurich, Switzerland.

**These authors contributed equally to this work.*

The atrioventricular canal (AVC) myocardium is essential for the delay of the electrical impulse from the atria to the ventricles in the developing heart, and defects in AVC maturation result in the most common forms of congenital heart disease. We sought to determine the role of canonical Wnt signaling in the myocardium during AVC development utilizing a novel allele of β-catenin that preserves β-catenin's cell-adhesive functions but disrupts canonical Wnt signaling, allowing us to probe the effects of Wnt loss of function independently. Loss of canonical Wnt signaling in the embryonic myocardium results in developmental defects along the spectrum of AVC defects, while ectopic activation of Wnt signaling in ventricular myocardium leads to ectopic constrictions and ion channel dysregulation consistent with formation of ectopic AV junctions. Although aberrant AV canal development can result in ventricular preexcitation, a characteristic feature of Wolff-Parkinson-White (WPW) syndrome, loss of canonical Wnt signaling alone was not sufficient to induce ventricular preexcitation. We previously described a mouse model that recapitulates many aspects of WPW syndrome via ectopic activation of Notch signaling within ventricular and AV canal myocardium, resulting in accessory pathway formation, upregulation of Scn5a expression, and cellular electrophysiological changes. In the current study, we demonstrate that postnatal activation of Notch signaling downregulates canonical Wnt targets, as well as bone morphogenetic proteins and Tbox transcription factors, within the AV junction. Stabilization of β-catenin protein levels can rescue Notch-mediated ventricular preexcitation and Scn5a upregulation, demonstrating a requirement for canonical Wnt inhibition in this genetic model of preexcitation. We propose that ventricular preexcitation requires developmental patterning defects giving rise to accessory pathways, as well as myocardial lineage reprogramming to allow robust conduction across accessory pathway tissue. These results suggest that myocardial Wnt and Notch signaling cooperatively regulate embryonic and postnatal AV canal maturation and myocardial cellular programming.

ASCI-27

Hedgehog-dependent Desmoplasia and Cancer-associated Fibroblasts Inhibit Growth and Progression of Pancreatic Ductal Adenocarcinoma: Implications for Targeted Therapy

***Andrew D. Rhim*[1], Paul E. Oberstein[2], Emily T. Mirek[3], Carmine F. Palermo[2], Steve A. Sastra[2], Erin N. Dekleva[3], Tyler Saunders[4], Christine Iacobuzzio-Donahue[4], Kenneth P. Olive[2], and Ben Z. Stanger[3]**

[1]Division of Gastroenterology, Department of Internal Medicine and Pancreatic Cancer Center, University of Michigan Medical School, Ann Arbor, MI; [2]Division of Hematology and Oncology and Division of Digestive and Liver Diseases in the Department of Medicine and Department of Pathology, Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center, New York, NY; [3]Gastroenterology Division, Department of Medicine and Abramson Family Cancer Research Institute, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA.; [4]Sol Goldman Pancreatic Cancer Research Center and Department of Pathology, Johns Hopkins University, Baltimore, MD

Sonic hedgehog (Shh), a soluble ligand overexpressed by neoplastic cells in pancreatic ductal adenocarcinoma (PDAC), drives formation of desmoplasia, a dense, fibrotic stroma comprising activated myofibroblasts and inflammatory infiltrates. It has been postulated that this desmoplastic response supports cancer progression based largely on experiments utilizing xenograft models in which resultant tumors tend not to share many of the features of human PDAC. To better understand its role in malignant progression, we deleted Shh in a well-defined genetically engineered mouse model of PDAC. As predicted, Shh-deficient tumors lacked desmoplastic stroma. Surprisingly, such tumors were more aggressive and exhibited undifferentiated histology (linked to worse prognosis in humans), increased vascularity, a shift to anabolic metabolism, and heightened proliferation — features that were fully recapitulated in control mice treated with a Smoothened inhibitor. Furthermore, administration of VEGFR2 blocking antibody arrested growth of only Shh-deficient tumors, indicating that hedgehog-driven desmoplasia suppresses tumor growth through effects on vasculature. Analysis of human PDAC reveals a tight correlation with undifferentiated histology, lack of desmoplasia, and enhanced vascularity. In summary, these data suggest that the desmoplastic tumor microenvironment, comprising cancer-associated fibroblasts and stroma, significantly inhibits tumor growth and progression, in part due to inhibitory effects on tumor angiogenesis and tissue perfusion. Our results also indicate that tumor differentiation status may be dependent upon a single signaling pathway in the pancreas. Finally, as undifferentiated human PDACs are highly vascular, unlike differentiated PDACs, tumors with these features may be susceptible to VEGFR inhibition. These data form the basis of a planned clinical trial to determine whether anti-angiogenesis agents may be effective in patients with tumors with undifferentiated histology identified on pre-treatment biopsy.

Young Physician-Scientist Award Abstracts

ASCI-28

Attenuated TGF-β Signaling Confers Insensitivity to TGF-β in dgkζ-deficient T Cells

Vidhyalakshmi Arumugam[1], T. Bluemn[1], Subramaniam Malarkannan[1,2], and *Matthew Riese*[1,2]

[1]Blood Research Institute, Blood Center of Wisconsin, Milwaukee, WI; [2]Division of Hematology/Oncology, Department of Medicine, Medical College of Wisconsin, Milwaukee, WI

Diacylglycerol kinase ζ (dgkζ) is a negative regulator of T cell receptor (TCR) signal transduction that metabolizes the second messenger diacylglycerol (DAG), terminating DAG-mediated activation of Ras and PKCθ. Cytotoxic T cells deficient in dgkζ demonstrate enhanced effector functions in vitro, along with increased activity against tumor in vivo. We recently determined that dgkζ-deficient T cells are insensitive to TGF-β, a pleiotropic cytokine that potently inhibits cytotoxic T cells, especially within the tumor microenvironment. The purpose of these studies was to determine the mechanism of TGF-β resistance in dgkζ-deficient T cells. Since TGF-β has been established to attenuate TCR signal transduction at a point upstream of DAG generation, we initially hypothesized that enhanced DAG-mediated signaling in dgkζ-deficient T cells provided relief from the inhibitory effects of TGF-β on TCR signaling, thus conferring functional resistance to TGF-β. However, analysis of TGF-β-induced transcriptional changes in dgkζ-deficient cytotoxic T cells revealed attenuation of all TGF-β-influenced genes examined, not solely those downstream of TCR, suggesting that TGF-β signaling was impaired globally. Subsequent biochemical evaluation of TGF-β signaling identified normal levels of TGF-β cell surface receptor but impaired nuclear translocation of Smad2/3, transcription factors responsible for mediating the TGF-β transcriptional program. We found that dgkζ-deficient cytotoxic T cells expressed increased baseline levels of Smad7, an antagonist of Smad2/3 activation and nuclear translocation, and decreased levels of cbl-b, the protein responsible for Smad7 degradation, whose degradation itself is regulated by PKCθ. Thus, we propose a model by which dgkζ-deficient T cells demonstrate resistance to TGF-β via increased degradation of cbl-b, decreased degradation of Smad7, and direct attenuation of Smad2/3 signaling. These data uncover dgkζ as a novel regulator of TGF-β signaling in cytotoxic T cells and suggest that the loss of dgkζ results in a broad set of functional consequences that lead to enhanced tumor clearance.

ASCI-29

Polyfunctional CD1b-restricted T cells Target Mycobacterial Glycolipids in South African Adolescents with Latent Tuberculosis Infection

***Chetan Seshadri*[1], Thomas J. Scriba[2], Glenna Peterson[1], David Freidrich[3], Nicole Frahm[3], Steven R. DeRosa[3], D. Branch Moody[4], Martine Gilleron[5], Hassan Mahomed[2], Saheli Datta[3], Lynn Lin[3], Greg Finak[3], Raphael Gottardo[3], Willem A. Hanekom[2], M. Juliana McElrath[3], and Thomas R. Hawn[1]**

[1]Department of Medicine, Division of Allergy and Infectious Diseases, University of Washington, Seattle, WA; [2]South African TB Vaccine Initiative, Cape Town, South Africa. [3]Vaccine and Infectious Disease Division and HIV Vaccine Trials Network, Fred Hutchinson Cancer Research Center, Seattle, WA; [4]Department of Medicine, Division of Rheumatology, Allergy, and Immunology, Brigham & Women's Hospital, Boston, MA; [5]Institute of Pharmacology and Structural Biology, CNRS, Toulouse, France

Most vaccines against *Mycobacterium tuberculosis* (MTB) focus on T cell responses to secreted proteins presented in the context of MHC molecules. However, the lipid-rich cell wall of mycobacteria also contains glycolipid antigens that T cells recognize in the context of CD1b molecules. The frequency and phenotype of MTB glycolipid-specific T cells have not been systematically assessed, and it is not known whether there is a correlation with T cell responses against immunodominant protein antigens. We developed an assay to profile polyclonal CD1b-restricted T cells from cryopreserved blood and performed a cross-sectional study of South African adolescents with and without latent tuberculosis infection. We assessed T cell responses to five mycobacterial glycolipids and five immunodominant proteins using multiparameter flow cytometry. T cell responses to glycolipids were more frequent within the double-negative and $CD8^+$ compared with the $CD4^+$ T cell subset. Responding $CD8^+$ T cells were more often polyfunctional than $CD4^+$ cells and were preferentially detected among subjects with latent tuberculosis. By contrast, polyfunctional T cell responses to protein antigens were detected almost exclusively within the $CD4^+$ T cell subset. Among the five lipids tested, glucose monomycolate induced the highest frequency of polyfunctional T cell responses. Thus, mycobacterial glycolipids induce T cell responses that are qualitatively different from T cell responses against immunodominant secreted proteins. Further study is warranted to assess the utility of glycolipids as immunogens for diagnostics and vaccines.

Young Physician-Scientist Award Abstracts

ASCI-30

Endoplasmic Reticulum Stress in the Lung as a Novel Mechanism for the Induction of Rheumatoid Arthritis

Oscar Estrada[1], Birthe Jessen[1], Christopher Law[1], Neil Trivedi[1], Jeremy Sokolove[2], and *Anthony Shum*[1]

[1]University of California San Francisco, San Francisco CA; [2]Stanford University, Palo Alto, CA

We performed whole exome sequencing of a family with a rare autosomal dominant form of rheumatoid arthritis (RA) and highly penetrant interstitial lung disease (ILD). We found a single, rare, non-synonymous variant in the surfactant protein A2 (SFTPA2) gene present only in diseased subjects. Mutations in the SFTPA2 gene are associated with familial pulmonary fibrosis and believed to cause disease through the induction of endoplasmic reticulum (ER) stress. However, none of the reported descriptions of families with SFTPA2 mutations include RA or autoimmunity as a clinical manifestation. In this family, RA is the dominant clinical phenotype in patients harboring the SFTPA2 mutation and in some patients the only manifestation of their disease. Interestingly, observational studies have shown that cigarette smokers that express the HLA shared epitope have a significantly elevated risk for the development of RA. The mechanism for this association remains unknown, but some propose that cigarette smoke promotes citrullination of proteins in the lung that in genetically susceptible hosts initiates RA. We recently confirmed that our patients express the HLA shared epitope. Thus, we hypothesize that the SFTPA2 mutation in this family results in lung protein citrullination and ultimately RA disease through the induction of ER stress. This novel family with RA-ILD allows us to dissect the relationship between ER stress and lung protein citrullination to uncover potential mechanisms for the observed association among cigarette smoking, the HLA shared epitope, and the risk of developing RA disease.

ASCI-31

β-Cell-derived miR-21 as an Intrinsic Protective Response and Biomarker in Type 1 Diabetes

***Emily K. Sims*[1,2], Ivan Restrepo[1,2], Xin Tong[2,3], Tatsuyoshi Kono[2,4], Raghu Mirmira[1,2,4], and Carmella Evans-Molina[2,4,5]**

[1]Department of Pediatrics, Section of Endocrinology and Diabetology, Indiana University School of Medicine, Indianapolis, IN; [2]Herman B. Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, IN; [3]Department of Cellular and Integrated Physiology, Indiana University School of Medicine, Indianapolis, IN; [4]Department of Medicine, Section of Endocrinology and Metabolism, Indiana University School of Medicine, Indianapolis, IN; [5]Richard L. Roudebush VAMC, Indianapolis, IN

A critical need exists for novel type 1 diabetes (T1D) treatment approaches focused on identification and intervention before widespread destruction of pancreatic β cells. Recent data suggest the intrinsic β cell response to inflammatory and metabolic stress ultimately impacts cell survival. Cytokine-induced inflammation increases miR-21 expression within the β cell. miR-21 targets the tumor suppressor phosphatase and tensin homolog deleted on chromosome 10 (PTEN) to increase cell survival in other tissues, but this relationship within the β cell remains unexplored. We hypothesized that cytokine-induced transcription of miR-21 by the β cell acts as a compensatory mechanism to repress PTEN translation in an attempt to limit β cell death. Furthermore, we predicted that stressed β cells release increased miR-21 compared with controls. INS-1 β cells were treated with a cytokine mix to mimic the early inflammatory milieu of T1D. RNA isolation, reverse transcription, and qRT-PCR were performed to analyze miR-21 expression, and immunoblotting was performed to quantitate PTEN levels. β Cell miR-21 expression was increased by 6 hours of treatment and peaked at 24 hours. β Cell PTEN protein levels were decreased at 24 and 48 hours. Expression of miR-21 in treated cell media was increased by 6 hours after cytokine exposure, reaching significance after 24 hours of treatment. This effect was magnified in the exosomal fraction of media. These results suggest an association between β cell production and release of miR-21 and decreased β cell PTEN protein levels in this model of early T1D. Future studies will evaluate direct relationships of miR-21 and PTEN in the β cell using miR-21 mimics and inhibitors, and luciferase assays, and in vivo studies will utilize a β cell-specific knockout mouse and human serum samples. Our results identify a probable relationship between miR-21 and PTEN within the β cell and miR-21's potential as a serum biomarker or treatment modality for early T1D.

ASCI-32

The Diabetes Susceptibility Gene Clec16a Regulates Mitophagy

***Scott A. Soleimanpour*[1,2], Aditi Gupta[1], Alana M. Ferrari[1], David N. Groff[1], João Fadista[3], Lynn A. Spruce[4], Jake A. Kushner[5], Leif Groop[3], Steven H. Seeholzer[4], Brett A. Kaufman[6], Hakon Hakonarson[7,8], and Doris A. Stoffers[1]**

[1]Division of Endocrinology, Diabetes and Metabolism, Department of Medicine and the Institute for Diabetes, Obesity and Metabolism of the University of Pennsylvania Perelman School of Medicine, Philadelphia, PA; [2]Division of Metabolism, Endocrinology & Diabetes and Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI; [3]Lund University Diabetes Center, Department of Clinical Sciences, Diabetes & Endocrinology, Skåne University Hospital, Lund University, Malmö, Sweden; [4]Children's Hospital of Philadelphia Research Institute, Philadelphia, PA; [5]McNair Medical Institute, Pediatric Diabetes and Endocrinology, Baylor College of Medicine, Houston, TX; [6]Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA; [7]Center for Applied Genomics, The Children's Hospital of Philadelphia, Philadelphia, PA; [8]Department of Pediatrics, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA

Clec16a has been identified as a disease susceptibility gene for type 1 diabetes, multiple sclerosis, and adrenal dysfunction, but its function is unknown. Utilizing high-throughput analysis of protein interaction partners found during immunoprecipitation and mass spectrometry, we report that Clec16a participates in a multi-subunit protein complex to regulate expression of Parkin, a master regulator of mitophagy. Islets from mice with pancreas-specific deletion of Clec16a have abnormal mitochondria with reduced oxygen consumption and ATP concentration, both of which are required for normal β cell function. Indeed, pancreatic Clec16a is required for normal glucose-stimulated insulin release. Furthermore, we find that Clec16a is a membrane-associated

endosomal protein that regulates pathways downstream of Parkin and its targets, including autophagosomal flux and ER homeostasis. Moreover, patients harboring a diabetogenic SNP in the Clec16a gene have reduced islet Clec16a expression and reduced insulin secretion. Thus, Clec16a controls β cell function and prevents diabetes by controlling mitophagy. This novel pathway could be targeted for prevention and control of diabetes and may extend to the pathogenesis of other Clec16a- and Parkin-associated diseases.

ASCI-33

Comparison of the Immunogenicity of Various Inactivated Polio Vaccine Booster Doses by Intradermal Versus Intramuscular Routes in HIV-infected Subjects

***Stephanie B. Troy*[1], Diana Kouiavskaia[2], Julia Siik[1], Efrat Kochba[3], Hind Baydoun[1], Olga Mirochnitchenko[2], Yotam Levin[3], Nancy Khardori[1], Konstantin Chumakov[2], and Yvonne Maldonado[4]**

[1]Eastern Virginia Medical School (EVMS), Norfolk, VA; [2]Center for Biologics Evaluation and Research, Food and Drug Administration (FDA), Rockville, MD; [3]NanoPass Technologies Ltd., Nes Ziona, Israel; [4]Stanford University School of Medicine, Stanford, CA

Background: Inactivated polio vaccine (IPV) will be needed globally for polio eradication because oral polio vaccine (OPV) can mutate into neurovirulent strains capable of causing poliomyelitis outbreaks, and because OPV may fail to provide adequate immunity in certain immunocompromised populations. However, IPV is currently unaffordable for many developing countries. Intradermal (ID) administration of IPV shows promise as a means to decrease the effective dose and thus the cost of IPV, but prior studies of ID IPV, all using 1/5 the intramuscular (IM) dose, showed inferior antibody titers compared with full-dose IM IPV. **Methods:** We conducted a randomized controlled clinical trial among 231 adults (mean age 46 years, mean CD4 count 645 cells/mm^3) with well-controlled human immunodeficiency virus (HIV) infection. Subjects were randomized to receive 2/5 dose ID IPV (66 subjects), 1/5 dose ID IPV (66 subjects), full-dose IM IPV (66 subjects), or 2/5 dose IM IPV (33 subjects). ID vaccination was done using the NanoPass MicronJet600 microneedles device. Serum collected before and one month after vaccination were analyzed by the FDA for polio-neutralizing antibody titers. **Results:** Baseline immunity in our subjects was 87%, 90%, and 66% against polio serotypes 1, 2, and 3, respectively. After vaccination, immunity was 99.6%, 99.6%, and 98.7%, and antibody titers rose a median of 64-fold, with no significant differences between study groups. Geometric mean titers were highest after vaccination in the 2/5 dose ID IPV group for all serotypes, but the differences between groups were not significant. **Conclusions:** HIV-infected adults on antiretroviral therapy still have significant immunity against polio an average of 40 years after childhood vaccination and demonstrate a robust memory response to booster vaccination. A booster of 2/5 ID IPV appears to result in antibody titers at least as high as full-dose IM IPV in HIV-infected adults.

ASCI / AAP Joint Meeting

2014

Abstracts for Oral Presentations and Poster Session

APSA

American Physician Scientists Association

www.jointmeeting.org

Oral Presentations

1

Ryanodine-Receptor Mediated Calcium Leak Drives Progression of Atrial Fibrillation

***David Y. Chiang*[1], Na Li[1], Sufen Wang[3], Qiongling Wang[1], Niels Voigt[4], Miguel Valderrabano[3], Stanley Nattel[5], Dobromir Dobrev[4], Xander H.T. Wehrens[1,2]**

[1]Department of Molecular Physiology and Biophysics; [2]Department of Medicine (Cardiology), Baylor College of Medicine, Houston, Texas; [3]Department of Cardiology, The Methodist Hospital, Houston, Texas; [4]Institute of Pharmacology, Faculty of Medicine, University Duisburg-Essen, Essen, Germany; [5]Department of Medicine, Montreal Heart Institute and Université de Montréal, Montreal, Quebec, Canada.

Background: The progression of atrial fibrillation (AF) from paroxysmal to persistent forms remains a major clinical challenge. Abnormal sarcoplasmic reticulum (SR) Ca^{2+} leak via the ryanodine receptor type-2 (RyR2) has been observed as a source of ectopic activity in various AF models. However, its potential role in progression to long-lasting spontaneous AF (sAF) has never been tested. This study tested the hypothesis that enhanced RyR2-mediated Ca^{2+} release underlies the development of a substrate for sAF. **Methods and Results:** CREM-IbΔC-X transgenic (CREM) mice developed age-dependent progression from spontaneous atrial ectopy to paroxysmal and eventually long-lasting AF. The development of sAF in CREM mice was preceded by enhanced diastolic Ca^{2+} release, atrial enlargement and marked conduction abnormalities. Genetic inhibition of CaMKII-mediated RyR2-S2814 phosphorylation in CREM mice normalized open probability of RyR2 channels and SR Ca^{2+} release, delayed the development of spontaneous atrial ectopy, fully prevented sAF, suppressed atrial dilation and forestalled atrial conduction abnormalities. Hyperactive RyR2 channels directly stimulated the Ca^{2+}-dependent hypertrophic pathway NFAT/Rcan1-4, suggesting a role for the NFAT/Rcan1-4 system in the development of a substrate for long-lasting AF in CREM mice. **Conclusions:** RyR2-mediated SR Ca^{2+} leak directly underlies the development of a substrate for sAF in CREM mice, the first demonstration of a molecular mechanism underlying AF progression and sAF substrate development in an experimental model. Our work demonstrates that the role of abnormal diastolic Ca^{2+} release in AF may not be restricted to the generation of atrial ectopy, but extends to the development of atrial remodeling underlying the AF substrate.

2

A Conditional NF2 Mouse Model of Schwannoma Genesis That Recapitulates Human Disease

***JR Gehlhausen*, SJ Park, MA Shew, CW Yates, DW Clapp**

Indiana University School of Medicine, Indianapolis, IN

Neurofibromatosis type 2 (NF2) is a genetic disease caused by inherited heterozygosity of the *NF2* tumor suppressor gene. NF2 patients exhibit progressive loss of hearing and vestibular function resulting from the development of bilateral vestibular schwannomas, which are pathognomonic for the disease. These patients also suffer from multiple spinal schwannomas that eventually cause a variety of neurologic deficits, including paralysis and autonomic impairment. In order to better study the molecular and cellular etiology of the pathologic manifestations observed in NF2 patients, we generated a new murine model that utilizes Cre Recombinase under the control of the *Postn* promoter to conditionally ablate *Nf2* in neural crest-derived cell populations. Histological analysis of the dorsal root ganglion (DRG) and proximal spinal nerves of *Postn-Cre+;Nf2*$^{flox/flox}$ mice revealed that these mice spontaneously develop tumors between six and eight months. Microscopic analysis implicated these tumors as schwannomas, as they display a pattern of dense celluarity consistent with the established criteria of GEM schwannoma, including S100 positivity. Quantitation of the DRG volume in ten mice indicated that *Postn-Cre+* mice have a five-fold increase in the size of their DRG. Auditory brainstem response (ABR) testing has revealed that *Postn-Cre+* mice develop progressively increased ABR thresholds by the age of eight months when compared to age-matched *Cre*-negative controls. This apparent hearing loss becomes more severe at ten months. Histological analysis of CN VIII confirmed that the mice developing hearing loss also have vestibular schwannomas. To test for concomitant vestibular loss of function, three different behavioral studies (contact righting, trunk curl, and swim) were performed to isolate and test the integrity of the vestibular system in *Postn-Cre;Nf2*$^{flox/flox}$ mice. *Postn-Cre;Nf2*$^{flox/flox}$ mice had profound difficulty with each test when compared to *Cre*-negative controls. Collectively, these genetically engineered mice acquire a phenotype that closely recapitulates important features of human NF2 disease and provides opportunities for testing putative therapeutic targets using genetic intercrosses or novel small molecule inhibitors.

Poster Abstracts

1

Neonates with Cystic Fibrosis Have a Reduced Nasal Liquid pH: A Small Pilot Study

MH Abou Alaiwa**[*]*****,* AM Beer[*], AA Pezzulo[*], JL Launspach[*], RA Horan[*], DA Stoltz[*], TD Starner[*], MJ Welsh[*†], J Zabner[*]**

[*]University of Iowa, Iowa City, IA; [†]Howard Hughes Medical Institute, Iowa City, IA

Background: Cystic Fibrosis (CF) is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) anion channel that conducts both Cl^- and HCO_3^-. Airway disease characterized by bacterial infection and inflammation remains the major cause of morbidity and mortality. Several observations suggest that disrupted HCO_3^- transport and reduced airway surface liquid (ASL) pH in cystic fibrosis (CF) may contribute to the pathogenesis of CF airway disease. We hypothesized that ASL pH is reduced in neonates with CF. **Methods:** We studied 28 neonates screened positive for CF with an immunoreactive trypsinogen test. We studied infants before results of their genetic tests or sweats tests were known. Thus, operators were unaware of the diagnosis. We measured the pH of nasal ASL within 3 months after birth. We also measured nasal pH in older children and adults. **Results:** In neonates with CF, nasal ASL (pH 5.2±0.3) was more acidic than in non-CF neonates (pH 6.4±0.2) ($p<0.01$). In contrast, the nasal pH of CF children and adults was similar to values measured in people without CF. **Conclusions:** At an age when infection, inflammation and airway wall remodeling are minimal, neonates with CF had an acidic nasal ASL compared to babies without CF. The CF:non-CF pH difference disappeared in older individuals, perhaps because secondary manifestations of the disease increase ASL pH. These results aid understanding of CF pathogenesis and suggest opportunities for therapeutic intervention and monitoring of the disease.

2

House Dust Mite-Induced Allergic Airway Disease is Associated with Reduced Regulatory Immune Cells in a Murine Model of Sickle Cell Disease

***AJ Adami,* SJ Bracken, LA Guernsey, B Andemariam, RS Thrall**

University of Connecticut Health Center, Farmington, CT

Introduction: Sickle cell disease (SCD) is associated with a constellation of pathologic changes that disturb every organ system. The pulmonary consequences of SCD are particularly severe and include high infectious burden, pulmonary hypertension, and increased severity of allergic asthma. Increased asthma severity in SCD may arise from the profound immunologic dysfunction characteristic of the disease. However, the precise immunologic mechanisms underlying asthma severity in SCD remain to be explored, particularly in the context of a clinically-relevant allergen such as house dust mite (HDM). The purpose of this study was to explore these immunologic disturbances using an HDM-induced murine model of asthma in transgenic SCD mice. **Methods**: Acute allergic disease was induced in transgenic SCD mice (University of Alabama at Birmingham strain, JAX #013071) and littermate control mice (WT) by intranasal challenge with 25 µg of HDM extract 5 days per week for 2 weeks. Following sacrifice, cells were isolated from bronchoalveolar lavage fluid (BAL), lung tissue, and the hilar lymph node (HLN) and stained for flow cytometric analysis. **Results**: The proportion of CD4+Foxp3+ regulatory T cells (Tregs) was significantly reduced in SCD mice versus WT controls in the BAL (8.84% vs. 14.0% of CD4+ T cells, $p < 0.05$) and lung (8.98% vs. 13.9% of CD4+ T cells, $p < 0.01$), but did not significantly differ in the HLN. Furthermore, the proportion of CD5+ B cells in SCD mice was nearly half that of WT controls in the BAL ($p < 0.0001$), lung ($p < 0.01$), and HLN ($p < 0.05$). Intriguingly, this CD5+ B cell population includes a group of regulatory B cells (Bregs) that our laboratory has shown to play an important role in the disease processes of murine asthma. **Conclusions**: Induction of allergic airway disease in SCD mice is associated with a reduced proportion of both regulatory T cells and the subset of B cells known to contain regulatory B cells. The dearth of regulatory cells in SCD may influence the increased severity of allergic asthma see in SCD. Treatment of asthma in SCD could be enhanced through efforts to target these cellular populations. ***Funding**: NIAID AI-043573 (RST), the Lea's Foundation Center for Hematologic Disorders (RST), and a Connecticut Institute for Clinical and Translational Science K12 Award (BA).*

3

The Importance of the RAGE Signal Cascade in the Mitigation of Nociceptive Neuronal Excitation

***Allette YM,* Ripsch M, White FA**

Indiana School of Medicine, Indianapolis, IN

Recent studies indicate that the release of high mobility group box 1 (HMGB1) following nerve injury may play a central role in the pathogenesis of neuropathic pain. HMGB1 is known to influence cellular responses within the nervous system via two distinct receptor families; the receptor for advanced glycation end products (RAGE) and toll-like receptors (TLRs). The degree to which HMGB1 activates a receptor is thought to be dependent on the redox state of amino acids C23 and C45 in Box A (disulfide HMGB1) and amino acid C106 within Box B (all thiol HMGB1). Though both receptor types are present on sensory neurons and can elicit neuronal activation *in vitro*, we show that neurons exposed to all thiol HMGB1 only acts through RAGE, while disulfide HMGB1 is restricted to TLR4. Moreover, RAGE mRNA and protein expression in lumbar dorsal root ganglion (DRG) is substantially increased following tibial nerve injury (TNI) by post-injury day (PID) 28 suggesting a possible role for all thiol HMGB1 and RAGE. To determine the degree of receptor influence on a rodent model of neuropathic pain behavior, we subjected injured rats to intraperitoneal injections of saline or a humanized monoclonal antibody to RAGE (RAGE Ab). A single exposure to RAGE Ab did not affect TNI-induced mechanical hyperalgesia on PID7-21. However, RAGE Ab administration on PID 28 produced a dose-dependent reversal of mechanical hyperalgesia. These results suggest that latent increases of RAGE signaling exhibit an influence on the maintenance of the neuropathic pain state and may contribute to a better understanding of mechanisms associated with chronic pain syndromes.

Poster Abstracts

4

Induced Changes in Tumor-Associated Macrophages and Tumor Malignancy in a Mouse Model of Sleep Apnea

Isaac Almendros**[*]**, **Yang Wang[*], Lev Becker[*], Frances E. Lennon[†], Jiamao Zheng[*], Brittney R. Coats[*], Kelly S. Schoenfelt[*], Alba Carreras[*], Fahed Hakim[*], Shelley X. Zhang[*], Ramon Farré[‡] and David Gozal[*]**

[*]Department of Pediatrics, Comer Children's Hospital, Pritzker School of Medicine, The University of Chicago – Chicago, IL; [†]Department of Medicine, University of Chicago – Chicago, IL; [‡]Universitat de Barcelona-IDIBAPS-CIBERES, Barcelona, Spain

Background: Increased cancer mortality and tumor aggressiveness have been recently associated with obstructive sleep apnea (OSA). Intermittent hypoxia (IH), a hallmark of OSA, enhances melanoma growth and metastasis in mice. **Objective:** To assess whether IH-induced tumor malignancy can occur through IH-induced changes in tumor-associated macrophages (TAMs). **Methods:** 40 B6 male 7-wk old mice were pre-exposed 2 weeks to either IH or room air (RA). 10^5 lung epithelial tumor cells (TC1) were injected in the left flank. After 4 weeks, TAMs were quantified in tumors by flow cytometry. Changes of phenotype were evaluated by protein surface proteomics and FACS analyses. Furthermore, TAMs were isolated from tumors in vivo (mice exposed to IH or RA) and their effects on some malignant properties of tumor cells were assessed in vitro. **Results:** Tumors from mice exposed to IH showed accelerated growth and invasiveness in comparison to RA-exposed mice. Increased malignant tumor behavior was accompanied by reductions in M1 polarity and a shift toward M2 pro-tumoral phenotype in IH-exposed TAMs. Proteomic analyses showed a marked decrease in IFIT1, IFIT3, TAP1, and TAP2 (proteins involved in the interferon response and antigen processing and presentation, respectively) in TAMs isolated from IH-exposed mice. Similarsly, surface expression of CD86 and CD40 (M1 markers) were reduced. In vitro studies confirmed TAMs functional changes. Co-culture of TAMs from tumors harvested from RA-exposed mice increased TC1 migration and extravasation. However, TAMs from IH-exposed mice markedly enhanced such effects (~ 2-fold), and also increased the proliferation (~50%) and invasiveness (~30%) of TC1 cells. Conclusions: Our findings support the notion that IH-induced alterations in TAMs underlie, at least in part, the adverse cancer outcomes reported in OSA. Supported in part by the Herbert T Abelson Endowed Chair in Pediatrics and Beatriu de Pinós fellowship from Generalitat de Catalunya (2010 BP_A 00238).

5

Neighborhood Social Composition and HIV/STI Risk Behaviors among Latino Migrant Men

MD Althoff**[*†]**, **K Theall[*], M Thompson[‡], H Gebrekristos[*], N Schmidt[*], P Kissinger[*]**

[*]Tulane University School of Public Health & Tropical Medicine, New Orleans, LA; [†]Tulane University School of Medicine, New Orleans, LA; [‡]University of New Orleans, New Orleans, LA

Background: The objective of this study is to determine role of neighborhood social composition, as measured by concentrated disadvantage and immigration concentration, on HIV and sexually transmitted infection (STI) risk behaviors among Latino migrant men (LMM). **Methods:** Three samples of LMM were combined to form a convenience sample of 251 participants. Data on participant demographics, social capital measures, past month HIV/STI risk behaviors were collected. Concentrated disadvantage and immigration concentration were calculated using factor regression weights from a factor analysis of census tract level estimates including: poverty, unemployment, female headed households, on public assistance, less than 18 years old, Black, Latino, foreign born, and speaks English less than very well. Data were obtained from the American Community Survey 5-year estimates. To determine each participant's neighborhood social composition buffers of varying radii were created around each participant's home address and a weighted average of the census tracts contained in the buffer was calculated. Logistic regression with robust standard errors to account for spatial clustering of participants was used to determine the relationship between concentrated disadvantage and HIV/STI risk behaviors. **Results:** Participants lived in areas with a higher median concentrated disadvantage and immigration concentration compared to all census tracts. Immigration concentration was not statistically associated with any HIV/STI risk behaviors. After adjusting for confounding, living in an area with higher concentrated disadvantage was associated with sex with a female sex worker, odds ratio (OR) 3.18 (1.22, 8.26), high risk sex OR 2.43 (1.15, 5.15), binge drinking OR 1.84 (1.01, 3.33), and drug use OR 4.03 (1.42, 11.44). Social support significantly mediated concentrated disadvantage and drug use. **Conclusions:** In this convenience sample of LMM living in the New Orleans area, men were living in areas with high immigrant concentration and concentrated disadvantage. The low variability of immigration concentration between participants likely contributed to the non-significant relationships with HIV/STI risk behaviors. There was, however, variability in concentrated disadvantage, which does appear to play a role in increased risk behaviors in this population, possibly by acting through decreased availability of social support.

7

A Bacterial-Derived Novel Lipid with Implications in the Disease Pathogenesis of Multiple Sclerosis

EJ Anstadt**[*]**, **F Nichols[†], R Nemati[‡], V Farrohki[‡], J Cervantes[*], M Fujiwara[*], RB Clark[*]**

[*]Department of Immunology, Department of Medicine, University of Connecticut School of Medicine, Farmington, CT; [†]Department of Oral Health and Diagnostic Sciences, University of Connecticut School of Dental Medicine, Farmington, CT; [‡]Department of Chemistry, University of Connecticut, Storrs, CT

Introduction: Multiple Sclerosis (MS) is a devastating disease affecting many people worldwide. The role of the microbiome in MS is inconclusive. We have characterized a novel Pathogen-Associated Molecular Pattern and TLR2 ligand, called Lipid 654, which is produced by many commensal bacteroidetes species in the GI and oral tracts. We found that Lipid 654 can be recovered in human serum and the levels are significantly lower in MS patients compared to healthy controls. The purpose of this study was to translate our human findings into murine models to better study the potential role of Lipid 654 in the pathogenesis of MS. **Aims:** Endogenous Lipid 654 levels in the serum, spinal cord, and brain

in wild-type mice with and without experimental autoimmune encephalomyelitis (EAE) were measured. Additionally, the effect of Lipid 654 treatment on disease progression and severity in EAE was determined. **Methods:** Lipids were extracted from the serum, spinal cord, and brain samples and the levels of Lipid 654 were determined using MRM-mass spectrometry. To purify Lipid 654 for injection into mice, total lipids were extracted from lyophilized *Porphyromonas gingivalis* and purified using HPLC and confirmed as Lipid 654 using MRM-mass spectrometry. The effect of treatment with 2 ug of Lipid 654 on EAE disease score was measured. **Results**: Naïve mice contained significantly higher levels of endogenous Lipid 654 in all tissue samples compared to mice with EAE. EAE disease severity was significantly reduced with Lipid 654 treatment. **Conclusions:** Endogenous Lipid 654 levels are highest in naïve mice, paralleling our healthy control population in the human studies. Lipid 654 treatment decreases EAE disease severity. Future directions will consist of creating a monoclonal antibody to Lipid 654 to carry out trafficking studies of Lipid 654 in mice, as well as aid in studies to determine the functional relevance of endogenous Lipid 654 in EAE through depletion. We will additionally further examine the mechanism of inhibition seen during treatment with Lipid 654 in EAE by studying potential immunoregulatory cells and cytokines that differ after treatment.

8

Characteristics of Long-term Survivors in Glioblastoma

C A Bridge[*1], *A L Baldock[2]*, P Kumthekar[1], P Dilfer[1], S K Johnston[3], J Jacobs[1], D Corwin[1], L Guyman[1], R Rockne[1], A Sonabend[4], M Cloney[4], P Canoll[4], K R Swanson[1]

[1]Northwestern University Feinberg School of Medicine, Chicago, IL; [2]UCSD School of Medicine, San Diego, CA; [3]University of Washington School of Medicine, Seattle, WA; [4]Columbia University College of Physicians and Surgeons, New York, NY

Intro: Glioblastoma multiforme (GBM) remains a devastating disease with a dismal prognosis. Only 2-5% of patients survive 3 years past diagnosis. Overall survival (OS) is related to younger age, higher initial Karnofsky Performance Status (KPS), complete surgical resection, MGMT promoter methylation and IDH positivity, but little else is known about long term survivor (LTs) tumor characteristics. **Methods:** To better understand underlying LTS characteristics, we examined 31 primary GBM patients with OS ≥ 3 years and compared them to a control cohort (OS < 500 days (N=162)). We evaluated tumor location, tumor size on MRI, presence of cyst at diagnosis (distinct from central necrosis), extent of resection, KPS and patient age. **Results**: No significant differences were observed with respect to tumor location, or tumor size at diagnosis between all cohorts. LTS displayed higher initial KPS (p=0.0127) and were younger at diagnosis (mean age=62.04 vs 53.10, p=0.00401). A larger proportion of LTS received gross total resections (56.7% vs 24.8% control patients, p=0.0005, Pearson Chi Square). LTS (N=31) had a higher percentage of cystic tumors (45% LTS, 24% control, p=0.0047). In the complete cohort (including 500<survival<1095, n=267), those with imageable cysts had longer OS (log rank test, p= 0.022). Age, KPS, cyst, and net diffusion rate estimated from MRI, are significant to survival (Cox PH, univariate, p=2.078e-08, 4.626e-08, 0.0279, 0.0012 respectively). Given these factors, location in the parietal lobe is significant to survival (Cox PH, multivariate, p=0.0202). **Conclusion:** To our knowledge, this analysis is the largest study to date of imaging characteristics and clinical features in long-term survivors of GBM. Our findings recapitulate previously identified attributes of LTS and demonstrate that cystic changes on pre-treatment MRI may also portend for long term survival. Further analysis of this population to elucidate if underlying phenotypic characteristics influence LTF is warranted.

9

AMPK Activation Attenuates Exercise Intolerance in Mice with Peripheral Vascular Insufficiency

***KA Baltgalvis,* K White, DG Payan, TM Kinsella**

Rigel Pharmaceuticals, Inc., South San Francisco, CA

Intermittent claudication is a form of exercise intolerance characterized by muscle pain during walking in patients with peripheral artery disease (PAD). Endothelial cell and muscle dysfunction are thought to be important contributors to the etiology of this disease, but a lack of preclinical models that incorporate these elements and measure exercise performance as a primary endpoint has slowed progress in finding new treatment options for these patients. We sought to develop an animal model of peripheral vascular insufficiency in which microvascular dysfunction and exercise intolerance were defining features. We further set out to determine if pharmacological activation of 5' AMP-activated protein kinase (AMPK) might counteract any of these functional deficits. Mice aged on a high-fat diet demonstrate many functional and molecular characteristics of PAD, including the sequential development of peripheral vascular insufficiency, increased muscle fatigability, mitochondrial dysfunction, and progressive exercise intolerance. These changes occur gradually and are associated with alterations in nitric oxide bioavailability. Treatment of animals with an AMPK activator increased voluntary wheel running activity, decreased muscle fatigability and prevented the progressive decrease in treadmill exercise capacity. These functional performance benefits were accompanied by improved mitochondrial function, the normalization of perfusion in exercising muscle, increased nitric oxide bioavailability and decreased circulating levels of the endogenous endothelial nitric oxide synthase (eNOS) inhibitor, asymmetric dimethylarginine (ADMA). These data suggest that aged, obese mice represent a novel model for studying endothelial cell and muscle dysfunction associated with peripheral vascular insufficiency, and pharmacological activation of AMPK may be a suitable treatment for intermittent claudication associated with peripheral artery disease.

Poster Abstracts

10

Directed Evolution of AAV9 Capsids for Enhanced Gene Delivery to the CNS After Systemic Injection

***AK Banerjee*†*, B Simpson†, P Pravdo†, BE Deverman†, and PH Patterson†

**Keck School of Medicine of USC, Los Angeles, CA; †California Institute of Technology, Pasadena, CA*

Multiple Sclerosis is a neurodegenerative disorder characterized by autoimmune destruction of the oligodendrocyte-derived myelin sheath. While remyelination by endogenous oligodendrocyte progenitor cells (OPCs) may occur in some patients, this process is highly variable. One therapeutic approach therefore, is to enhance this reparative process through the stimulation and proliferation and/or differentiation of endogenous OPCs and neural stem cells (NSCs) towards the production of new oligodendroglia. Our group previously showed that targeted delivery of Leukemia Inhibitory Factor (LIF) to the central nervous system (CNS), through administration of a *LIF*-encoding adenovirus, stimulates NSC self-renewal and OPC proliferation. Given the high immunogenicity of adenovirus-based vectors, new viral-vectors are currently being investigated and developed, including those based on Adeno-associated virus (AAV). AAV9-based vectors are particularly attractive for the treatment of neurodegenerative disorders because of their ability to cross the blood brain barrier (BBB) following intravenous injection. Although AAV9 is able to cross the BBB and transduce neurons and glia in the adult mouse, the efficiency of transduction may be too low to provide sufficient therapeutic benefit. Since the AAV capsid is responsible for determining tissue tropism and transduction efficiency, efforts have been made to engineer novel AAV capsid variants with desired biologic properties. Modification of AAV capsids based on sequence analysis and peptide insertion has met limited success. In contrast to these rational-design based approaches, directed evolution and *in vivo* screening can be used to generate and select for novel AAV variants with altered biologic properties, including increased resistance to neutralizing antibodies or altered cell tropism. We have developed a novel screening approach that selectively recovers capsid sequences that transduce the desired target cell population and are using this approach to screen several AAV9-based capsid libraries to identify variants that more efficiently transduce CNS astrocytes.

11

VE-Cadherin Mechanotransduction Regulates Lung Endothelial Contractility and Barrier Integrity

***AK Barry*[1,2], N Wang[3], DE Leckband[4,5]

[1]Department of Biochemistry; [2]Medical Scholars Program; [3]Department of Mechanical Engineering; [4]Department of Chemical and Biomolecular Engineering; [5]Department of Chemistry, University of Illinois at Urbana-Champaign, Urbana, IL

Dysfunctional regulation of endothelial adherens junctions is the direct underlying cause of vascular leak and pulmonary edema in acute lung injury. Indirect evidence suggests that cytoskeletal rearrangements are linked to adherens junction remodeling, but there is no clear demonstration that adherens junction proteins play an active role in the regulation of intracellular or extracellular mechanical tension. Here I investigate mechanotransduction (response to applied force) of VE-cadherin, the main structural protein at adherens junctions, and how this regulates global cell contractility and actin remodeling both locally and globally. Mechanical responses in human pulmonary artery endothelial cells were studied using magnetic twisting cytometry (MTC) experiments, in which shear stress was exerted on specific cell surface receptors by twisting magnetized beads bound to the cell surface. Remodeling of adherens junctions and F-actin in response to mechanical force was visualized by immunofluorescence. Confocal microscopy revealed force-actuated changes in both local and global cytoskeletal organization, as well as local rearrangements at both bead-cell and cell-cell junctions. This force-dependent remodeling correlated directly with the stiffening of VE-cadherin junctions with increasing applied stress. Treatment with cytoskeletal inhibitors significantly diminished this response. When a constant magnitude of shear stress was applied continuously over time, VE-cadherin junctions increased stiffness, suggesting active junction reinforcement. These data provide direct evidence that VE-cadherin complexes are tension sensors, and that associated mechanotransduction regulates global cell mechanics. Moreover, I present data showing that cadherin adhesions across the cell monolayer form a mechanically coupled network that regulates the endothelial barrier in response to force. This suggests a new mechanism regulating endothelial monolayer integrity.

12

Paradoxical Effects of NKG2D Ligands on NK Anti-Tumor Immunity

***F Basher**, S Lu, G Xiao, J Wu

**Department of Microbiology & Immunology, Medical University of South Carolina, Charleston, SC*

Many human tumors of epithelial origin express abundant levels of ligands for the NK activating receptor NKG2D but still progress to poorly differentiated carcinomas, indicating alteration of NKG2D-mediated active immune surveillance. Several clinical studies indicate that levels of NKG2D ligand expression correlate with disease prognosis in less aggressive cancers but are inversely correlated in more aggressive or later staged cancers. Shedding of the soluble NKG2D ligand MIC is one method by which tumor cells escape immunosurveillance to promote cancer progression. Previous studies in our laboratory using a humanized bi-transgenic prostate adenocarcinoma model showed that tumor progression to metastasis correlated with serum soluble MIC levels similar to the patient population. Interestingly, significant deficits in NK cell homeostasis and cytotoxic activity were also observed in the presence of soluble MIC. The aims of this study are (1) to identify mechanisms responsible for impaired peripheral NK cell homeostasis and function in the presence of soluble MIC and (2) to investigate whether these defects can be rescued with synergistic therapies aimed at neutralizing MIC and promoting NK function. *In vitro* co-cultures of NK cells with a TC2 cell line expressing soluble MIC significantly increased NK cell exhaustion, demonstrated by PD-1 expression, by 114% ($p<0.01$) as well as decreased phospho-AKT and phospho-STAT5, components of NK survival and activation pathways, by 80% and 76% respectively ($p<0.01$), compared to NK cells cultured with TC2 cells expressing

membrane-bound MIC. An *in vitro* therapy model demonstrated combined treatment with PD-1 and MIC blocking antibodies reduces soluble MIC-induced NK PD-1 expression to baseline levels. Rag -/- mice inoculated with TC2 cells expressing soluble MIC in an *in vivo* therapy study and treated with IL-15/IL-15Ra/Fc, neutralizing antibody against MIC, or the combination had significantly decreased splenic apoptotic (total annexin V+ and annexin V+/PD-1) frequency (65% and 74% respectively, $p<0.05$) as well as decreased the frequency of micrometastases in the lung (43%, $p<0.01$). These studies indicate a role for PD-1-mediated NK exhaustion with chronic stimulation by the soluble ligand MIC and possible NK cell rescue and potentiation by the use of combination therapies involving IL-15 stimulation, PD-1 pathway blockade, and neutralization of soluble MIC.

13

2-Dimensional Simulation of the Actin Comet Tail of Listeria Using Queueing Theory

***RO Bauer*, BJ Yi**

**Department of Mathematics, University of Illinois at Urbana-Champaign and College of Medicine, University of Illinois at Urbana-Champaign*

Background: Once Listeria monocytogenes invades a cell it exploits the dynamics of the intracellular actin network for its propulsion and movement into adjacent cells. Not having to leave the cytosol as it moves from cell to cell allows the pathogen to evade the humoral immune response. A functional, quantitative description of these dynamics that can be effectively simulated and implemented on a computer can improve our understanding of how this pathogen spreads in tissues and suggest *and then test* avenues for possible pharmacological counter measures. We have developed simulations for Listeria's propulsion in the cytosol by modeling the dynamics of the actin cytoskeleton as a network of 'queues' or 'waiting lines.' Actin filaments are dynamic directed polymers where 'free' actin monomers from the cytosol can attach themselves at one end and actin monomers 'bound' to the polymer can dissociate from the other end. In terms of queueing theory, actin monomers are 'customers,' and actin filaments are 'queues' or 'waiting lines' in front of a server whose service provided is 'dissociation.' Customers arrive at one end of the queue and leave from the other after the service has been provided. We model this behavior on a planar triangular lattice where each actin monomer corresponds to a directed edge connecting two neighboring vertices on the lattice. A linear string of edges corresponds to an actin filament. New edges arrive at the +-end of the filament at an exponential rate α, and dissociate from the minus end at rate β. Different filaments are treated as stochastically independent. The pathogen is modeled as a large hexagon with a distinguished vertex (the 'nucleation pole') on the lattice and is pushed forward and rotated according to an appropriate algorithm when a filament that touches the pathogen grows. In its simplest form the model depends on three parameters, α, β, and N (the size of the hexagon). Using results from queueing theory, the distribution of the length of a filament, the maximal length of a filament, and the lifetime of a filament can be calculated. Simulation of this stochastic process produces movement of the hexagon that is similar to the movement of listeria observed in cells. Parameters that describe the erratic nature of the path the hexagon follows, e.g. what is the typical length of straight-line movement of the hexagon before it changes direction, can be matched with statistics for the observed behavior of Listeria.

14

Specificity and Promiscuity in the ComRS System of Gram-Positive Pathogens

ER Beisner*, MJ Federle*

**Center for Pharmaceutical Biotechnology, University of Illinois at Chicago, Chicago, IL*

Background: Cell-to-cell signaling in bacteria, also known as quorum sensing, involves the production and detection of small molecules that are exchanged between bacteria. Quorum sensing is used to coordinate important physiological processes such as virulence development, biofilm production, and the ability to transfer genetic information between cells, also called competence. Defining these signaling mechanisms and developing methods to interfere with them could provide novel strategies to treat bacterial infections. *Streptococcus mutans*, the primary agent of dental caries, uses a quorum sensing pathway involving a short peptide pheromone called XIP and its cognate receptor, ComR. XIP develops from a precursor peptide termed ComS that is secreted from the cell and processed into the mature pheromone. XIP is transported into the cytosol where it is bound by ComR. The ComR/XIP complex induces a transcriptional cascade that results in development of the competent state. Interestingly, the ComRS pathway and all known components of the competence system are conserved across all members of the Mutans, Pyogenic and Bovis groups. Using an *S. mutans* 'test bed' strain that we developed to contain a luciferase reporter monitoring ComR activity, we have tested interactions of con- and hetero-specific pairings of XIP and ComR alleles. Our data indicate that although XIP orthologs are generally specific to each species, there is evidence of cross-talk between species and groups of streptococci. By characterizing the interactions between all combinations of ComR and ComS proteins, we have gained evidence as to what is necessary for a successful interaction. Examination of these XIP amino acid sequences and ComR gene sequences will aid our abilities to define components of XIP that are critical to generate a productive signal, and will also allow us to begin developing methods to interfere with other related pheromone-dependent signaling pathways in Gram-positive bacteria.

15

Integrative Genomic Analysis of the Responses to Naturally Acquired Acute Respiratory Virus Infection

***JW Belmont*[1,6], Y Zhai[1,4], R Atmar[2,3], J Quarles[4], N Arden[4], J Wells[3], D Niño[3], L Franco[1,2], R Couch[2,3]**

[1]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; [2]Department of Medicine, Baylor College of Medicine, Houston, TX; [3]Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX; [4]Department of Microbial and Molecular Pathogenesis, Texas A&M University System Health Science Center, College Station, TX

Objectives: Acute viral respiratory infections (ARIs) are responsible for huge numbers of outpatient visits and hospitalizations in the U.S. each year. In this study we are characterizing functional gene networks correlated with clinical and immune responses

to infection. **Methods:** 1618 healthy adults were prospectively enrolled in fall 2009 and 2010. Subjects reporting moderate ARI symptoms had virus quantitation for 3 weeks. Peripheral blood RNA samples were obtained at enrollement and then on the first day of illness (day 0) as well as 2, 4, 6, 21 days after first symptoms. RNA samples were analyzed using expression microarrays and differentially expressed transcripts identified. DNA was used for genome wide SNP genotyping. Sera were analyzed for humoral immune response. **Result:** Among the 133 subjects who completed 5 illness day study visits, 73 had influenza infection, 64 influenza A and 9 influenza B. Twenty-four had at least one other known viral respiratory pathogen e.g. human rhinovirus (HRV) or respiratory syncytial virus (RSV). Gene expression profiles showed highly similar patterns among the major subgroups. There was a dramatic up-regulation of interferon pathway and innate immunity on the first day of infection. A convalescent phase was observed on days 4 and 6 after infection. By day 21 the gene expression pattern had returned to baseline. Using lineage specific transcripts to produce cell composition scores, patterns of acute depression of lymphocytes were observed accompanied by the evidence of intense activation of dendritic cells and NK cells. Coherence of gene networks increased significantly during the acute phase of infection. The strength of the network coherence correlated with clinical severity score. Thousands of cis eQTL were observed with a subset showing substantial increase in genetic effect in the context of infection. **Conclusions:** An integrative genomic approach identifies gene networks and genetic variants that contribute to individual variation in response to viral infections. Transcriptional profiling gives a genome wide view of a coordinated systemic response to acute viral respiratory infection. There are two clear phases of gene expression, corresponding to intense activation of innate immunity pathways followed by a convalescent phase marked by cell proliferation and repair.

16

Activity and Expression of the Protective Angiotensin Converting Enzyme 2 is Increased in the Rat Brain Following Ischemic Stroke

D Bennion, **E Haltigan, C Kilar, A Irwin, C Sumners**
University of Florida, Gainesville, FL

Purpose: While activation of the classical renin angiotensin system is known to exert deleterious increases in stroke risk, morbidity, and mortality, a newly discovered counter-regulatory axis of this system, known as the angiotensin converting enzyme 2 – angiotensin-(1-7) – mas [ACE2–Ang-(1-7)–Mas] pathway, has been shown to be neuroprotective in stroke. Until very recently, the impact of stroke on the expression levels of the components of this axis was unknown. In this study, we assessed the hypothesis that in the 24 hours following ischemic stroke, expression and enzymatic activity of the ACE2 would be increased in a time-dependent fashion. **Methods:** Adult male Sprague-Dawley rats underwent sham surgery or ischemic stroke by endothelin-1 induced middle cerebral artery occlusion followed by euthanasia at +4, +12, or +24h after stroke (n=4 per group). Levels of ACE2 activity were assessed in the infracted cortex using an established fluorometric assay. Expression of ACE2 mRNA was determined using qRT-PCR. Data are mean ± SEM.

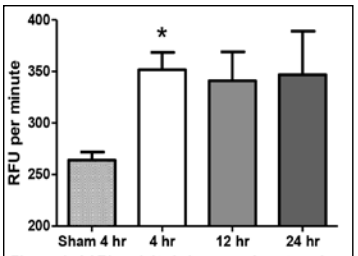


Figure 1. ACE2 activity is increased post-stroke. Following induction of ischemic stroke, groups of rats were euthanized at the time points indicated, and tissue homogenate from the infarcted cortex was assayed for enzymatic peptidase activity of ACE2 using a specific fluorogenice petpide substrate. RFU, relative fluorescence unit. *p<0.05 versus Sham 4 hr.

Results: At 4h post-stroke, ACE2 activity levels in the ischemic cortex were significantly increased (351.8±16.67, p=0.013) as compared to sham stroke (263.9±7.94, **Fig 1**). Further, there was a significant (p=.032) four-fold increase in expression of ACE2 mRNA in infracted cortex from 12h to 24h after stroke. **Conclusions:** Our findings suggest that stroke induces increased activation and expression of the protective ACE2–Ang-(1-7)–Mas axis, further implicating the brain renin-angiotensin system as a promising target for translational stroke research.

18

A Mouse Model of Autoimmune Narcolepsy

R. Bernard-Valnet*, **B. Pignolet****, **C. Peyron†, R. Liblau****
**INSERM U1043, CNRS, UMR 5282, Centre de physiopathologie de Toulouse Purpan, Toulouse, France; †INSERM U1028, CNRS, UMR 5292, Center for Research in Neuroscience of Lyon, Team SLEEP, Lyon, France*

Narcolepsy with cataplexy is a rare and severe disabling sleep disorder. It is characterised by irresistible daytime sleepiness and sudden loss of muscle tone called cataplexy. Histopathological studies have shown that narcolepsy is caused by a destruction of neurons of hypothalamus secreting orexin (also termed as hypocretin). Even though the mechanisms of this disease are still unknown, converging evidences suggests an autoimmune process. First, there is a strong association with HLA-DQB1*0602 allele (95% of patients vs 25% in western countries population) and with polymorphisms in the T cell receptor α locus. Secondly, the vaccination campaign for pandemic swine flu in 2009 has been associated with a dramatic increase of the occurrence of childhood narcolepsy suggesting a putative environmental trigger. However the experimental models of narcolepsy are currently based on genetic invalidation of the orexin system of the hypothalamus and do not allow studying aetiology of this disease. Our goal is to generate a new mouse model in order to study narcolepsy pathophysiology with a specific focus on the immune mechanisms leading to orexin neurons loss. The experimental approach is to develop mice expressing influenza virus hemagglutinin (HA) in the orexin neurons of the hypothalamus and to induce an autoimmune destruction of these neurons by the injection of effectors T lymphocytes specific for HA. Thanks to this model we would be able to study: the migration of T cells in the hypothalamus, their ability to selectively destroy orexin neurons, the key player of these processes. We would also be able in a second part to assess the impact of vaccination or infection on triggering narcolepsy hallmarks. Our transgenic mice (Orex-HA) are expressing HA in more than 75% of orexin neurons. The injection of in vitro differentiated CD8 cytotoxic T lymphocytes specific for HA, will lead to a massive infiltration of CD3+ cells in the hypothalamus. The pick of this infiltrate is around 8 days after injection and slow down after with a focalisation in orexin neurons area. This was associated with a local but massive activation of microglial cells. No such infiltration was seen in Littermate

animals. This infiltration and activation of immune cells was associated by loss of orexin neurons including axonal budding. This loss was up to 75% in Orex-HA mice compared to controls. No such destruction was observed in surrounding neurons. We also investigated sleep phenotype in these mice. In this model, we provided evidences that CD8 cytotoxic lymphocytes are able to migrate in the hypothalamus and destroy specifically orexin neurons bearing a neo-self antigen. We now would like to investigate if the peripheral activation, by virus or vaccination, is able to lead to the same phenotype.

19

Comparison of the Protective Roles of *Trypanosoma cruzi*-specific Th1 and Th17 Cells

***JR Blase*[*], CS Eickhoff[*], DF Hoft[*]**

[*]St. Louis University, St. Louis, MO

Background: *Trypanosoma cruzi* is a protozoan parasite and the causative agent of Chagas disease. The *T. cruzi* protein trans-sialidase (TS) is currently under investigation as a vaccine candidate. Previous studies have shown that *T. cruzi*-specific Th1 cells are important for protective mucosal and systemic immunity. However, the role of Th17 cells in *T. cruzi* protective immunity has yet to be explored in detail. **Methods:** TCR-transgenic, TSaa57-74-specific $CD4^+$ T cells were purified from naïve transgenic mice and differentiated in vitro into Th1 or Th17 cells. In vitro, *T. cruzi*-specific Th1 and Th17 cells were co-cultured with *T. cruzi*-infected peritoneal exudate macrophages (PEMs), and protection was assessed microscopically by the presence of intracellular amastigotes (AMAs). Variations of this in vitro protection assay were used to determine possible mechanisms of T cell-mediated protection. In vivo, *T. cruzi*-specific Th1 or Th17 cells were adoptively transferred into *Rag-/-* mice along with naïve polyclonal $CD8^+$ T cells. Mice were challenged with *T. cruzi,* persistent immune responses were measured, and protection was assessed by parasitemia and survival. **Results:** In vitro, both Th1 and Th17 cells led to protection against intracellular *T. cruzi* growth in macrophages. Th1-mediated protection involves IFN-γ signaling to induce nitric oxide production by macrophages and subsequent killing of intracellular *T. cruzi* AMAs. Th17-mediated protection is dependent on IL-17A and independent of iNOS induction. We are currently exploring the role of reactive oxygen species generation, autophagy, apoptosis, and alternative means of lysosomal killing. Surprisingly and perhaps more importantly, in vivo, Th17 cells provided significantly better protective effects than Th1 cells. The mechanism for Th17-mediated protective effects in vivo involves enhanced expansion of pathogen-specific $CD8^+$ T cells. **Conclusion:** These results reveal that Th17 cells have both direct and indirect effects on *T. cruzi* protective immunity and demonstrate that Th17 cells can provide helper effects for $CD8^+$ T cells protective against an intracellular pathogen.

20

Global Downregulation of Exosomal microRNA in Mice with Diet-Induced Obesity

***IO Blokhin,* S Ramachandran, EJ Devor, PB McCray Jr., KK Leslie, SR Lentz**

University of Iowa Carver College of Medicine, Iowa City, IA

Obesity, which has reached epidemic proportions in developed countries, is associated with increased risk of cardiovascular events and malignancies, but the mechanisms underlying these associations are still poorly understood. MicroRNA (miR) are small non-coding RNA that, after processing by the specific nucleases *Drosha* and *Dicer*, induce post-transcriptional repression of target mRNA. A major fraction of plasma miR is contained within exosomes, which are small (20 to 90 nm) vesicles that can mediate intercellular transfer of nucleic acids. In the current study, we tested the hypothesis that diet-induced obesity (DIO) alters the miR composition of plasma exosomes in mice. To induce obesity, C57BL6/J mice were fed a 60% high-fat diet (TG.06414, Harlan Laboratories) for 12 weeks. Exosomes were immunoprecipitated from plasma, and the exosomal miR profile was determined using a Rodent TaqMan Low Density Array (TLDA) that detects 760 individual miR. *Drosha* and *Dicer* mRNA and protein levels were measured by real-time PCR and Western blotting. We found that DIO resulted in global repression of exosomal miR expression. Among 343 miR detected by TLDA, 317 (92%) demonstrated an obese-to-lean ratio ≤ 1, indicating lower levels in obese vs. lean mice, and only 26 (8%) had an obese-to-lean ratio of > 1. Using a P-value threshold of 0.05, we found that 41 miR were significantly repressed, and no miR were significantly increased, in obese mice. Bioinformatically predicted target genes of the 41 repressed miR clustered in 5 disease hubs, including obesity, heart failure, and three cancers. Expression of *Drosha* and *Dicer* mRNA was decreased in liver and adipose tissue from obese mice compared with lean mice, suggesting a potential mechanism of global miR downregulation in obesity. We conclude that diet-induced obesity induces a generalized (global) repression of miR expression in plasma exosomes. Since plasma exosomes can transport miR to target tissues, decreased exosomal miR content may represent one mechanism by which obesity enhances expression of genes involved in the pathogenesis of obesity-associated chronic disease.

21

Skeletal Muscle Denervation Causes Skeletal Muscle Atrophy through a Pathway that Involves Both Gadd45a and HDAC4

***KS Bongers*[*], DK Fox[*], SM Ebert[*], SD Kunkel[*], MC Dyle[*], SA Bullard[*,†], JM Dierdorff[*], CM Adams[*,†]**

[*]University of Iowa, Departments of Molecular Physiology and Biophysics and Internal Medicine and Fraternal Order of Eagles Diabetes Research Center, Iowa City, IA; [†]Iowa City Veterans Affairs Medical Center, Iowa City, IA

Denervation-induced skeletal muscle atrophy is a common secondary consequence of a variety of clinical conditions, including diabetes, amyotrophic lateral sclerosis, alcoholism, and Charcot-Marie-Tooth disease. However, despite its prevalence and

severity, the molecular mechanisms responsible for denervation-induced muscle atrophy remain incompletely understood. To better understand the molecular pathogenesis of this common, debilitating condition, we investigated the regulation of growth arrest and DNA damage-inducible 45a (Gadd45a) in denervated skeletal muscle. Previous studies demonstrated that muscle denervation induces Gadd45a mRNA expression, which in turn increases Gadd45a, a small nuclear protein that is required for denervation-induced muscle fiber atrophy. However, how denervation increases Gadd45a expression remained unknown. Our studies show that the lysine deacetylase HDAC4 is required for Gadd45a induction after muscle denervation. Conversely, HDAC4 overexpression increases skeletal muscle Gadd45a mRNA and causes muscle fiber atrophy in the absence of muscle denervation. Furthermore, Gadd45a mediates several important downstream effects of HDAC4, including induction of myogenin mRNA and, importantly, myofiber atrophy. Because Gadd45a is also a critical mediator of fasting-induced muscle atrophy, we next tested whether HDAC4 might regulate Gadd45a expression during fasting. Interestingly, however, HDAC4 is not required for Gadd45a mRNA induction or muscle atrophy after fasting. Additionally, the bZIP transcription factor ATF4, which is required for fasting-induced Gadd45a expression, does not contribute to denervation-induced Gadd45a expression or muscle atrophy. Taken together, these findings show that HDAC4 is an essential regulator of Gadd45a in muscle atrophy after denervation and identify Gadd45a as a convergence point for different upstream regulators during fasting- and denervation-induced muscle atrophy.

22

Alveolar Macrophages Transition to a Regulatory Phenotype Following Chronic Exposure to Inhaled House Dust Mite Antigen

Sonali J. Bracken, **Alexander J. Adami, Linda A. Guernsey, Steven M. Szczepanek, Roger S. Thrall**
University of Connecticut Health Center, Farmington, CT

Background: Allergic asthma is a leading cause of morbidity worldwide and results from lack of immunological tolerance to innocuous antigens. Alveolar macrophages (AMs) are among the first cells to encounter inhaled allergens and may play an important role in both the induction and resolution of allergic inflammation. The purpose of this study was to investigate the potential role of AMs in tolerance development using a murine model of house dust mite (HDM)-induced allergic airway disease (AAD). **Methods:** Wild type C57BL/6 and IL-10^{gfp} mice were challenged intranasally (i.n.) with 25 µg HDM extract 5 days/wk for 2 wks (acute; AAD phase) or 11 wks (chronic; resolution phase). Control animals received i.n. PBS. Upon sacrifice, cells isolated from bronchoalveolar lavage fluid (BAL) and lung tissue were stained for flow cytometric analysis. Pulmonary function was assessed via methacholine challenge. **Results:** Mice that were acutely exposed to HDM developed AAD, as noted by a significant increase in airway hyper-reactivity (AHR), BAL eosinophil frequency, and total serum IgE levels compared to control mice. Conversely, chronic HDM-exposed mice developed tolerance to HDM, as noted by resolution of AHR and BAL eosinophilia as well as a reduction in total serum IgE levels. Chronic HDM exposure was associated with a 9-fold increase in AMs compared to control mice and a 3-fold increase in AMs compared to acute HDM-exposed mice. Phenotypic analysis of these cells demonstrated a 3-fold reduction in frequency of CD206^{+} alternatively-activated AMs in chronic versus acute HDM-exposed mice. These cells have been strongly linked to the pathogenesis of AAD. Moreover, chronic HDM exposure in IL-10^{gfp} mice was associated with a significant increase in IL-10- and TGF-β-producing AMs that exhibited reduced MHCII expression, suggesting their transition from a pathogenic to a regulatory phenotype.**Conclusion:** Chronic HDM exposure stimulates AMs to transition from pro-inflammatory (i.e. AAD promoting) to regulatory cells. This suggests that AMs may play a crucial role in orchestrating tolerance to commonly inhaled allergens and may thus serve as a potential therapeutic target for patients with asthma.

This work was funded by: NIH/AI R01 HL-43573 (RST), T32AI007080 (SJB)

23

Surgical Implantation of uLED Devices for Optogenetic Nerve Blockade

DS Brenner[1,2,3], G Shin[4], T Kim[5], JA Rogers[4,6,7,8], RW Gereau IV[1,2]
[1]MD-PhD Program, Washington University in St. Louis, MO; [2]Department of Anesthesia, Washington University in St. Louis, MO; [3]Neuroscience Program, Washington University in St. Louis, MO; [4]Department of Materials Science and Engineering, Frederick Seitz Materials Research Laboratory, University of Illinois at Urbana-Champaign, IL; [5]School of Chemical Engineering, Sunkgkyunkwan University Korea; [6]Department of Electrical and Computer Engineering, University of Illinois at Urbana-Champaign, IL; [7]Department of Mechanical Science and Engineering, University of Illinois at Urbana-Champaign, IL; [8]Department of Chemistry, University of Illinois at Urbana-Champaign, IL

Pain is crucial issue, costing an estimated $560-635 billion annually in the United States alone. Despite this expenditure, recent studies have shown that pain is not adequately managed in a wide range of patient populations. In many conditions, anesthetic nerve blocks are an effective first line treatment; however, because these pharmacological interventions only provide transient relief and cause a number of undesirable side effects, they cannot be used for long-term relief. A non-pharmacological, long-term nerve block technique could significantly improve treatment for these patients. Optogenetics utilizes algae-derived light-sensitive ion channels to hyperpolarize or depolarize cells in order to inhibit or generate action potential firing, respectively. If these channels are expressed in sensory neurons, nocifensive responses can be generated or blocked by appropriate light exposure. This presentation will describe our work to develop novel, wireless, implantable µ-LED devices allowing implementation of optogenetics to generate long-term, modulatable nerve blocks in awake, behaving mice.

Supported by NINDS funds 1F31NS078852 to DB and an NIH Director's Transformative Research award (R01NS081707) to RG

Poster Abstracts

24

The Affordable Care Act's Impact on Translational Research & the Availability of Discretionary Federal Funding for the National Institutes of Health

CE Brokowski
Columbia University, New York, NY

Introduction: Health care constitutes 1/7 of the United States' gross domestic product, a value of approximately $2.241 trillion. In March 2010, The Patient Protection and Affordable Care Act and the Health Care and Education Affordability Reconciliation Act—together constituting the Affordable Care Act (ACA)—were signed into law. The ACA includes critical provisions: extending health insurance coverage to over 3 million people; ending lifetime limits on insurance coverage; and expanding Medicaid to cover households with up to 138 percent of the poverty level. Despite controversy regarding its constitutionality and policy implications, little attention has been given to the ACA's potential impact on translational research and the discretionary funding available for the National Institutes of Health (NIH). The purpose of this research was to investigate how key provisions of the ACA may influence the translational research milieu and NIH funding levels. **Methods:** The ACA was evaluated for stipulations pertaining to translational research. Also, linear regression and Pearson product-correlation coefficient analyses were conducted to i) determine whether the NIH appropriation level is positively or negatively correlated with the funding of the ACA; ii) examine the strength of this relationship, if any; iii) determine whether the NIH funding levels per institute are correlated with the funding of the ACA; & iv) examine the strength of these relationships. **Results:** First, 9 out of the 10 Titles in the Patient Protection and Affordable Care Act were found to have promising influence on translational research: Title I: Quality Affordable Health Care for All Americans; Title II: The Role of Public Programs; Title III: Improving the Quality and Efficiency of Health Care; Title IV: Prevention of Chronic Disease and Improving Public Health; Title V: Health Care Workforce; Title VI: Transparency and Program Integrity; Title VII: Improving Access to Innovative Medical Therapies; Title IX: Revenue Provisions; & Title X. Strengthening Quality, Affordable Health Care for All Americans. Second, statistical data regarding the aforementioned correlations (if any), and their strength, among funding levels of the NIH, its specific institutes, and the ACA, are pending. **Conclusions:** While many provisions of the ACA hold great promise to facilitate bench-to-bedside, translational research in the clinical arena especially, its funding mandate and position may result in competition with the NIH for discretionary monies from the federal appropriations process.

25

Dual Treatment with Tamoxifen and Trifluoperazine Inhibits Malignant Peripheral Nerve Sheath Tumor Proliferation and Survival More Effectively Than Monotherapy

Stephanie Brosius, **Amy Turk, Stephanie Byer, John Kappes, Andrew Carroll, Kurt Zinn, Kevin Roth, Steven Carroll**
University of Alabama at Birmingham, Birmingham, AL

Malignant peripheral nerve sheath tumors (MPNSTs) are aggressive sarcomas derived from cells in the Schwann cell lineage. These tumors carry a poor prognosis and represent the most common cause of death in patients with the genetic tumor susceptibility disorder neurofibromatosis type-1, largely due to the dearth of effective chemotherapeutic regimens. We have previously demonstrated that tamoxifen inhibits MPNST cell survival in an estrogen receptor-independent manner and also kills MPNST cells in an orthotopic xenograft model. To further investigate the effects of tamoxifen *in vivo*, NOD-SCIDγ mice were orthotopically grafted with ST88-14 MPNST cells and treated with a range of tamoxifen doses for 30 days, resulting in a maximum 50% reduction in graft size. Graft development did not require circulating endogenous sex hormones as evidenced by xenograft experiments in castrated or ovariectomized mice. Treatment with the calmodulin inhibitor trifluoperazine, also inhibited MPNST proliferation and survival. Therefore, we hypothesized that combinatorial treatment would tamoxifen and trifluoperazine would result in greater inhibition of graft growth compared to either monotherapy. To determine if tamoxifen and trifluoperazine act on distinct signaling cascades, we performed kinomic analyses in treated MPNST cells and some key signaling cascades (Akt and JNK) were affected by both agents, while others were uniquely affected by tamoxifen or trifluoperazines. To test the hypothesis that combinatorial treatment with tamoxifen and trifluoperazine was more effective than monotherapy, mice were grafted with either ST88-14 cells or STS-26T cells and treated with vehicle, half-maximal doses of tamoxifen or trifluoperazine, or a combination of the two drugs. Either individual treatment produced a maximum 50% inhibition of graft growth, while combinatorial treatment resulted in a 90% reduction in graft mass. Combinatorial treatment with tamoxifen and trifluoperazine decreased Ki67 labeling and increased apoptotic labeling relative to vehicle or single agent therapy. These studies provide a strong rationale for clinical trials in patients with sporadic or NF1 associated MPNSTs.

26

Laser Diffraction for Point-of-Care Detection of High-Level Loiasis: A Tool to Enable Elimination of Filarial Diseases

***PJ Budge*[1], DW Wright[2]**
[1]*Division of Infectious Diseases, Department of Medicine and*
[2]*Department of Chemistry, Vanderbilt University, Nashville, TN*

Background. Lymphatic filariasis (LF) and onchocerciasis (river blindness) are parasitic infections affecting hundreds of millions of persons worldwide and are two of the world's most debilitating infectious diseases. Mass drug administration (MDA) programs, in which all persons in areas endemic for LF or onchocerciasis are presumptively treated annually or every 6 months, are effective in eliminating these diseases, but receipt of MDA medications can cause fatal encephalopathy among persons infected with another filarial parasite, *Loa loa*. The risk of fatal encephalopathy increases with the level of *Loa* parasitemia and persons with high-level (>8,000 microfilaria/mL) loiasis must be excluded from MDA. As a result, MDA have been halted in *Loa*-endemic areas pending development of technology capable of diagnosing high-level loiasis at point-of-care during MDA campaigns, so that affected persons can be excluded from receiving MDA medications. **Methods and Results**. We are developing a low-cost, rapid assay for quantifying nematode larvae using *C. elegans* as a proxy

organism. A low-power HeNe laser beam is directed through a cuvette containing *C. elegans* L1 larvae, which are approximately the same size as *Loa* microfilaria, suspended in buffer solution. The thrashing motions of the larvae diffract the beam, and diffracted light is detected by a sensitive photodiode (Thorlabs DET10A) connected to an oscilloscope (PicoScope 2105). In preliminary experiments, red light (650 nm) gives a better signal-to-noise ratio than green light (523 nm) and is linear in the range of 25 – 500 L1s/mL (R^2=0.95); future experiments will characterize the linear range of the assay, optimize assay parameters, and adapt the assay to detection of filarial larvae in whole blood. **Conclusions**. A simple laser diffraction assay can quantitatively detect the motion of nematode larvae in solution and may provide the basis for a rapid, low-cost, point-of-care test for detecting high-level loiasis in the setting of MDA for LF and onchocerciasis.

27

Recombinant Melittin Protein Therapy for Treatment of High Grade Astrocytoma

Jason S. Buhrman, **Jamie E. Rayahin, Richard A. Gemeinhart**
University of Illinois at Chicago, Chicago, IL

Background: With a median survival of 15 months, grade IV astrocytoma, or glioblastoma, is among the most feared diagnosis in medicine, and its prognosis has remained largely unchanged. Melittin is a cell lytic peptide derived from the venom of the *Apis cerana* honeybee and has shown promise in glioblastoma treatment. Protein therapeutics are widely active in the body and are prone to aggregation and degradation with both processes leading to inactivation of the molecule. Hydrogels have shown promise in protecting proteins from extracellular hazards, but modes of reversible protein attachment to hydrogels are lacking. To this end, we developed a novel protein anchor based on natural affinity of the glutathione s-transferase (GST) to its cofactor glutathione (GSH). **Results:** We decorated poly (ethylene glycol) diacrylate (PEGDA) microspheres with pendent GSH, and found that the spheres could specifically interact GST fusion proteins (GST-GFP and GST-melittin). The interaction between the GST and GSH was stable both human plasma for at least one week. Upon thrombin treatment, proteins fused to GST are released from the spheres. GST-melittin not associated with microspheres shows decreased activity to U118 cancer cells, but when associated with the spheres shows no measurable activity. Melittin regains activity upon release from the microspheres. **Discussion:** As the number of known therapeutic peptides and proteins continues to increase, the need for viable delivery systems to increase their clinical utility is rising. Delivery systems require some mode of capturing active molecules until they are released. We demonstrate one such mode of immobilization with a fused anchor. We have shown that this anchor is stable in human plasma, and would expect it to be stable in most extracellular environments where reduced GSH levels are low. Conversely, we would expect this anchor to be less in the intracellular environment due to high levels of reduced GSH. Finally, such a protein anchor allows for the possibility of delivering multiple proteins at modulatable stoichiometries. Due to the ease of creation, and the already high numbers of therapeutic proteins being purified with GST fusion anchors, we believe the GST/GSH anchor to be a promising component of future protein delivery systems.

29

Local Iontophoretic Drug Delivery Device Improves the Administration of Gemcitabine for the Treatment of Pancreatic Cancer

JD Byrne*, **MR Jajja****, **A O'Neill****, **ME Napier****, **JC Luft****, **WC Zamboni****, **JJ Yeh****, **JM DeSimone*†‡**
**University of North Carolina at Chapel Hill, Chapel Hill, NC; †North Carolina State University, Raleigh, NC; ‡Memorial Sloan Kettering Comprehensive Cancer Center, New York, NY*

Pancreatic cancer is the fourth leading cause of cancer death in the world, with an incidence rate approximately equivalent to the death rate. The very poor prognosis for pancreatic cancer can be attributed to late diagnosis and the ineffectiveness of drug delivery to the primary tumor. Poor tissue perfusion plays a substantial role in preventing adequate drug exposure to primary pancreatic tumors. The systemic administration of gemcitabine, the current standard-of-care chemotherapy for pancreatic cancer, has shown limited efficacy for the treatment of pancreatic cancer, with only 5 to 10% of patients demonstrating an objective radiographic response at the primary tumor site. Furthermore, the toxicity associated with systemic chemotherapy has been found to reduce the quality of life of the patient and in extreme cases can be fatal. In an attempt to address the lack of effectiveness of systemically administered chemotherapy and associated toxicity, we have developed modalities for the localized delivery of chemotherapies to pancreatic tumors. These devices rely upon an applied electric potential difference between electrodes to drive chemotherapy into the tumor. This use of an electric potential for local delivery of chemotherapies offers the capability of overcoming considerable flow and pressure gradients. Significant work using an applied electric potential difference for drug transport has been performed in transdermal drug delivery. In the area of oncology, electric field-assisted delivery techniques have been proposed for skin and ocular cancers and have been clinically translated to the treatment of bladder cancers in the electromotive delivery of mitomycin C. This technique, combined with novel device approaches, is particularly well suited for the local treatment of the primary pancreatic tumor. Herein, we report the development of electric field-assisted delivery devices and the testing of these devices in a patient-derived orthotopic xenograft mouse model of pancreatic adenocarcinoma and a healthy canine animal model. The results show that our devices provide a significant increase in the local delivery and effectiveness of gemcitabine while limiting systemic toxicity.

30

Long Term Impairment of CD4 T Cell Responses After Sepsis

J Cabrera-Pérez*, **SA Condotta‡**, **BR James****, **D Rai‡**, **TA Kucaba****, **VP Badovinac‡**, **TS Griffith*†**
**U. of Minnesota, Minneapolis, MN; ‡U. of Iowa, Iowa City, IA; †VAMC, Minneapolis, MN*

Sepsis strikes 750,000 Americans every year; an estimated 210,000 of these patients die, making sepsis the leading cause of death in most intensive care units. Patients who survive the acute stages of sepsis often display severely compromised immune function; as a consequence, death in septic patients is oftentimes related

to subsequent nosocomial infections. CD4 T cells, which are essential for the coordination of successful immune responses to opportunistic pathogens, are severely depleted during the acute stage of sepsis and gradually recovered afterwards. However, little is known regarding the mechanism(s) behind this recovery or the extent to which sepsis impairs CD4 T cell function in surviving patients. Using a cecal-ligation and puncture (CLP) model to induce intra-abdominal peritonitis, we tracked endogenous, antigen-specific CD4 T cell populations throughout loss and recovery, and tested their responsiveness to a secondary infectious challenge. Our results demonstrate that a massive loss of CD4 T cells occurs acutely after sepsis induction, and that CD4 T cell recovery is quantitatively apparent 30d post CLP. After recovery, we observed an increased frequency of $CD44^{hi}CD11a^{hi}CD49d^{hi}$ CD4 T cells—a surface phenotype consistent with CD4 T cells that have encountered Ag (e.g. 'Ag-experienced'). However, the acquisition of this Ag-experienced phenotype was independent of cognate Ag recognition, given that adoptively-transferred, TCR-transgenic CD4 T cells specific for *S. typhymurium* flagellin (SM1 cells) or LCMV glycoprotein (SMARTA cells) displayed increased frequencies of Ag-experienced CD4 T cells as well. IL-7Rα expression was also higher in CD4 T cells from septic hosts when compared to surgical controls, and the frequency of Ag-experienced CD4 T cells was unchanged in $IL7^{-/-}$ hosts 30d after CLP. Finally, these phenotypic changes were independent of IL15 availability or thymic output. Despite numerically-apparent T cell recovery, we found a sustained impairment of Ag-specific CD4 T cell responses to a heterologous bacterial infection, persisting for as long as 30 days after sepsis induction and resolution. Using a p:MHCII tetramer enrichment approach, we found that the recovery of certain Ag-specific CD4 T cell precursor populations was incomplete in septic mice. Our results suggest that CD4 T cells undergo peripheral recovery by homeostatic proliferation after sepsis-induced lymphopenia, and that this is a phenomenon driven by IL-7 and self-Ag:MHCII availability in the system. Furthermore, an asymmetric or skewed recovery of the epitope-specific CD4 T cell repertoire contributes to persistently- impaired primary CD4 T cell responses. Our findings demonstrate that sepsis can result in substantial changes to the available CD4 T cell repertoire, affecting the capacity of the host to respond to newly introduced Ag (infections) long after the septic event has resolved.

31

Vascular Stem Cell Therapy of the Diabetic Retina with COMP-Ang1 and Endothelial Progenitor Cells

***Judd M Cahoon*[1], PR Olson[1], H Uehara[1], C O'Neill[2], R Medina[2], A Stitt[2], GY Koh, BK Ambati[1]**

[1]University of Utah, Salt Lake City, UT; [2]Queen's University Belfast, Belfast, Northern Ireland; [3]Korean Advanced Insitute of Science and Technology (KAIST), Daejeong, South Korea

Background: Diabetic retinopathy (DR) is the leading cause of blindness in the working-age population. Restoring vascular homeostasis and replacing lost endothelial cells (reparative angiogenesis) could reduce the neurovascular damage that occurs in DR. An important factor lost in diabetic retinopathy is Angiopoietin-1 (Ang1), which promotes endothelial survival and vascular stability. Outgrowth endothelial cells (OECs) are a specific subtype of endothelial progenitor cell that have the potential to reintegrate into damaged vascular beds and promote reparative angiogenesis. The purpose of this study was to determine whether a novel Ang1 analog, COMP-Ang1, could *prevent* the structural and functional hallmarks of DR and *reverse* diabetic damage by increasing OEC integration into the diabetic retina. **Methods:** Diabetic Ins2Akita mice were treated at 2 months (before the onset of DR) with a single intravitreous dose of adeno-associated virus (AAV2) encoding COMP-Ang1 (AAV2.COMP-Ang1) or control (AAV2.GFP). Four months later retinas were assessed for functional (Evans blue leakage), inflammatory (acridine orange leukography), and structural (confocal microscopy) status. A second cohort of 7 month-old mice (after the onset of DR) were treated with AAV2.COMP-Ang1 and labeled OECs (harvested from the mononuclear layer of donated cord blood). Three days later retinas were harvested and analyzed with confocal microscopy to assess OEC integration into the retinal vasculature. **Results:** AAV2.COMP-Ang1 preserved vascular structure in 6-month-old diabetic mice and decreased vascular leak and leukocyte adhesion. Furthermore, retinal thinning and ganglion cell layer dropout were prevented and treated mice retained near-normal visual acuity and electroretinographic response compared to controls. COMP-Ang1 increased OEC migration speed, tube formation, and Akt phosphorylation in a dose-dependent manner *in vitro*. In the second cohort of mice, preliminary results suggest that COMP-Ang1 increases OEC integration into the retinal vasculature compared to control. **Conclusions:** AAV2.COMP-Ang1 may be useful in preventing diabetic neurovascular dysfunction. OECs and COMP-Ang1 can play a functional reparative role in diabetic retinopathy and other diabetic microvascular diseases. Future studies will determine whether newly integrated OECs reduce functional deficits in DR.

32

Follistatin-like Protein 1 Regulates IL-17 Signaling by Influencing the Expression of IL-17 Receptor

***BT Campfield*[*], R Hirsch[+], JK Kolls[*]**

[*]University of Pittsburgh School of Medicine, Pittsburgh, PA; [+]University of Iowa, Iowa City, IA

Background: IL-17, the canonical Th17 cytokine, is critical for the development of Collagen Induced Arthritis (CIA) and pulmonary host defense. Follistatin-like protein-1 (FSTL-1), a poorly characterized proinflammatory glycoprotein, mediates the development of CIA in a T-cell dependent manner. The mechanisms by which FSTL-1 mediates inflammation are unknown. **Methods:** ST2 cells with shRNA for FSTL-1 (shFSTL-1) and control (shCntrl) sequences , or primary murine C57Bl/6 wild-type (WT) or FSTL-1 knock-out (FSTL-1 KO) bone marrow stromal cells (BMSC) were stimulated with 8ng/mL IL-17A and 2ng/mL TNFα. Transcript abundance was assessed by qRT-PCR. Protein secretion was assessed by Luminex. IL-17 receptor surface expression was assessed by FACS analysis. **Results:** Compared to ST2 shCntrl cells, shFSTL-1 cells had 1) decreased transcripts of IL-6 (275±3 vs. 43±3), G-CSF (1802±7 vs. 1325±93), IL-17RA (0.798±0.02 vs. 0.504±0.03) and IL-17RC (1.28±0.01vs. 0.72±0.1) relative to unstimulated cells, 2) decreased protein secretion IL-6 (2542 ± 37 vs 25142 ± 229pg/ml), G-CSF (27.8 ± 3.9 vs. 388 ± 32 pg/ml) secretion, and 3) decreased IL-17RA surface expression when unstimulated (52.3% vs. 37.2%) despite similar IL-17RC surface

expression when unstimulated (93.6% vs. 91.4%). Compared to WT primary BMSCs, FSTL-1 KO BMSCs had 1) decreased transcripts of IL-6 (664.5±13 vs.159.9±19), G-CSF (76.2±0.01 vs. 6.1±1.1), IL-17RC (1.30±0.03 vs. 0.17±0.05), but similar IL-17RA (1.22±0.04 vs. 1.23±0.27) relative to unstimulated cells, and 2) decreased IL-17RC surface expression when unstimulated (90.9% vs. 50.3%). **Conclusions:** FSTL-1 mediates IL-17 stimulated IL-6 and G-CSF production in the ST2 cell line and in primary bone marrow stromal cells. FSTL-1 influences transcript abundance of IL-17 receptor transcription and surface expression, suggesting that the effects of FSTL-1 on IL-17-mediated cytokine production occur at the level of the IL-17 receptor complex.

33

A Decrease in Mitochondrial Iron is Protective Against Ischemia/Reperfusion Damage

***H.-C. Chang*[1], R. Wu[1], H. Ardehali[1]**
[1]*Feinberg Cardiovascular Research Institute, Northwestern University. Chicago, IL*

Background: Excessive free iron can catalyze the formation of reactive oxygen species and cause cellular damage. Mitochondria are a major site of iron utilization, but the role of mitochondrial iron in ischemia/reperfusion (I/R) injury is unclear. We recently showed that mitochondria iron export is mediated by ATP-binding cassette protein B8 (ABCB8). **We hypothesize that mitochondrial iron has an important role in I/R damage and a decrease in mitochondrial iron is protective against I/R damage through a reduction of ROS.** **Results:** Cardiomyocyte-specific ABCB8 transgenic (TG) mice demonstrated a decrease of mitochondrial iron without impairing cardiac function at baseline comparing to nontransgenic (NTG) littermates. We subjected ABCB8 TG and NTG littermates to I/R to study the role of mitochondrial iron in I/R damage. ABCB8 TG mice displayed significantly better cardiac function and significantly reduced apoptosis after I/R compared to NTG littermates. In addition, ABCB8 TG mice have significantly reduced lipid peroxidation products compared to NTG mice, suggesting a ROS-related protection mechanism. To further confirm our mechanism, we utilize pharmacological method to reduce mitochondrial iron *in vitro*. 2,2-bipyridyl (BPD) is a mitochondria-accessible iron chelator while deferoxamine (DFO) has poor penetrance into mitochondria. Treating rat cardiomyoblasts H9C2 with BPD but not DFO significantly decreased mitochondrial iron and prevented H_2O_2-induced cell death. We are currently testing the protective effect of pharmacological intervention in *in vivo* I/R model. **Conclusions:** Our findings demonstrate that selective reduction in mitochondrial iron is protective in I/R injury, and show that mitochondrial iron is a source of ROS and cellular damage in I/R. Thus, targeting mitochondrial iron with selective iron chelators, as studied in our system, may provide a novel approach for treatment of ischemic heart disease.

34

Interleukin 22 Down Regulates ABCG1 and Impairs Cholesterol Efflux in Macrophages

***B Chellan*[*], L Yan[*], TJ Sontag[†], CA Reardon[†], MAH Bowman[*]**
[*]*Department of Medicine and* [†]*Department of Pathology; University of Chicago, Chicago, IL*

Background: IL-22 belongs to the IL-10 cytokine family and is expressed by T helper cells. IL-22 functions on epithelial cells and has been shown to improve epithelial barrier functions. IL-22 is induced locally in inflammatory bowel disease, asthma, and psoriasis. Patients with psoriasis have increased coronary artery disease and it was previously shown that macrophages from patients with psoriasis have impaired cholesterol efflux. The function of IL-22 on macrophage cholesterol metabolism is not known. **Methods:** ABCA1,ABCG1 and CD36 mRNA and protein expression, cholesterol uptake and efflux were studied in murine macrophages and human THP-1 macrophages. C57BL6/J mice with transgenic expression of S100A12 and S100A8/9 in myeloid cells were generated by using a bacterial artificial chromosome (hBAC/S100 mice). hBAC/S100 and WT littermates were bred into mice lacking receptor for advanced glycation end products, RAGE. **Results:** Peritoneal macrophages from hBAC/S100 mice had reduced ABCG1 mRNA and protein expression, increased cholesterol uptake and reduced cholesterol efflux compared to WT. This was abolished in hBAC/S100 mice lacking RAGE, the receptor for S100/calgranulin. Recombinant S100A12 or S100A8 protein (2.5ug/ml) had no effects on ABCG1 expression in WT peritoneal macrophages or human THP-1 cells suggesting other systemic intermediary products in hBAC/s100 mice. Plasma IL-22 and mRNA in bone marrow derived macrophages were increased in hBAC/S100 mice and this was abolished in mice lacking RAGE, Moreover, IL-22 mRNA increased by 2 fold in cultured human THP-1 cells in response to rS100A12. Importantly, THP-1 treated with rIL-22(100ng/ml) had reduced expression of ABCG1 and impaired cholesterol efflux to mouse serum (mostly HDL), but not ApoA1. Up regulation of ABCG1 and ABCA1 in response to LXR agonist TO901317 abolished the detrimental effects of IL-22 on cholesterol efflux. **Conclusion:** S100/calgranulin promotes secretion of IL-22 in a RAGE dependent manner. IL-22 down regulates ABCG1 and impairs cholesterol efflux in macrophages. This raises the hypothesis that elevated IL-22 associated with autoimmune diseases may improve epithelial barrier function via down regulation of cellular cholesterol efflux but thereby could possibly augment atherosclerosis.

35

The Gut-Liver Axis Plays a Reversible Role in Pulmonary Inflammation After Intoxication and Burn Injury

***MM Chen,* A Zahs, M Brown, L Ramirez, and EJ Kovacs**
Loyola University Chicago Stritch School of Medicine, Maywood, IL

Of the 450,000 burn patients each year in the US, nearly 50% are intoxicated at the time of injury and have worsened clinical outcomes compared to those without prior alcohol exposure. Pulmonary dysfunction is common in these patients and intoxication prior to burn has also been shown to increase

pulmonary inflammation in mice. Reduction of interleukin-6 (IL-6) in mice improves these pulmonary parameters, intestinal permeability and overall mortality. These animal studies parallel clinical observations of burn patients where elevated serum IL-6 correlate with mortality risk. The intestinal microbiome may influence diseases through interaction with the liver via the "gut-liver axis." Burns result in a loss of intestinal barrier function, allowing the movement of bacteria into the portal circulation. Alcohol also effects the gut-liver axis and intoxication prior to burn in mice leads to greater bacterial translocation from the intestines and more hepatosteatosis than either insult alone. Intoxication potentiating the ability of burns to cause acute liver injury is of significant clinical concern as the liver is a central player in regulating the over exuberant inflammatory response that is thought to be responsible for worsened prognosis. We hypothesize that intoxication alters the gut-liver axis leading to increased pulmonary inflammation mediated by IL-6 produced by the liver after burn. To this end, mice were given 1.2g/kg ethanol 30 minutes prior to a 15% total body surface area burn. To restore gut barrier function, a specific myosin light chain kinase inhibitor (membrane-permeant inhibitor of kinase (PIK)) was administered 30 minutes after injury which we have demonstrated reduces bacterial translocation from the gut. We found that intoxication exacerbates post-burn pulmonary inflammation and liver damage which is associated with a 4-fold increase in circulating IL-6 ($p<0.05$) and a 7-fold increase in hepatic IL-6 mRNA expression ($p<0.05$) compared to sham-injured mice. Limiting gut leakiness with PIK attenuated hepatic damage as measured by a 36% reduction in serum alanine aminotransferase ($p<0.05$) as well as a 57% reduction in hepatic IL-6 mRNA expression ($p<0.05$) compared to intoxicated burned mice without PIK. This mitigation of hepatic damage was associated with a 41% decline in pulmonary neutrophil infiltration ($p<0.05$), a 31% reduction in pulmonary KC and decreased alveolar wall thickening compared to matched controls without PIK. Overall these data suggest the gut-liver axis is deranged when intoxication precedes burn and that limiting bacterial translocation attenuates hepatic damage and pulmonary inflammation.

36

Lasting Antibody Responses Are Mediated by Immature and Long-Lived Bone Marrow Plasma Cells Drawn from Distinct Precursors

***Irene Chernova*[*], Alexandra Bortnick[*] and David Allman[*]**

[*]Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA

Plasma cells are the immune system cells responsible for producing antibodies, the key mediators of protective humoral immunity. Long-lived plasma cells (PC) are thought to be responsible for maintaining antibody titers and are believed to populate unique survival niches in the bone marrow (BM). Current models predict that bone marrow plasma cells (BM PC) consist chiefly of long-lived, slowly renewing cells. However, we show the turnover rate of the BM PC pool to be much higher than predicted by these models; in fact, more than 50% of BM PC exhibit characteristics of recently formed PC. Intriguingly, these B220$^+$ PC do not appear to be cycling and are depleted upon ablation of peripheral B cell pools. In extending our studies to antigen-induced responses, we find that very long-term maintenance of the antigen-specific BM PC pool is dependent on a CD40-independent B cell precursor. Despite the rapid turnover rate exhibited by B220$^+$ BM PC, antigen-induced antibody secreting cells are found within this population for more than 100 days post-immunization. These cells secrete exclusively low affinity, unswitched, □ type antibodies in sharp contrast to the high affinity, isotype switched cells found within the slowly renewing BM PC pool. Finally, we identify a population of rapidly renewing memory B cells that appear to be the precursors of the B220$^+$ BM PC. Together these data suggest that BM niches are continuously repopulated by newly generated plasma cells long after antigenic exposure and identify the memory B cell precursors of BM PC, drastically changing our conception of antibody titer maintenance mechanisms.

Funded by NIH AI-097590 and AI-090700.

37

β_2-Adrenergic Agonists Augment Air Pollution-Induced Thrombosis

***SE Chiarella*[*], S Soberanes[*], D Urich[*], L Morales-Nebreda[*], R Nigdelioglu[*], D Green[*], JB Young[*], N Foiles[*], A Gonzalez[*], CM Rosario[*], A Misharin[*], AJ Ghio[†], RG Wunderink[*], HK Donnelly[*], H Perlman[*], NS Chandel[*], GRS Budinger[*], GM Mutlu[*]**

[*]Northwestern University, Chicago, IL; [†]United States Environmental Protection Agency, Research Triangle Park, NC

Background: Exposure to particulate matter (PM) air pollution increases all-cause mortality by increasing the incidence of thrombotic cardiovascular events; however, the mechanisms explaining this association are incompletely understood. We have previously demonstrated that exposure to PM causes an enhanced tendency towards thrombosis sufficient to cause intravascular thrombin generation and accelerate arterial thrombosis after injury through an IL-6 and alveolar macrophage dependent mechanism. **Objective:** To determine if the activation of β_2-adrenergic receptors (β_2ARs) on alveolar macrophages is required for PM-induced release of IL-6 release and acceleration of arterial thrombosis. **Methods:** For *in vivo* experiments, we employed: (1) global knockout mice lacking the adrenergic receptors β_1, β_2, or both β_1 and β_2 with their wild-type littermate controls and (2) *LysM-Cre/*$\beta_2AR^{fl/fl}$ mice with their control mice ($\beta_2AR^{fl/fl}$). For *in vivo* PM exposure, we employed two different methods: (1) intratracheal instillation of a well-characterized PM collected from Washington, DC and (2) inhalation of concentrated ambient PM (CAPs), for measurement of catecholamines, IL-6, thrombin-antithrombin (TAT) complexes and carotid artery occlusion time after injury. For *in vitro* studies, we used MH-S cells, primary mouse alveolar macrophages and primary human alveolar macrophages, for measurement of catecholamines, mitochondrial reactive oxygen species (ROS), IL-6, cAMP, PKCδ and CREB. **Results:** We found that PM exposure results in the systemic release of catecholamines, which engage the β_2AR on human and murine alveolar macrophages to augment the release of IL-6. In mice, this promotes the development of a prothrombotic states sufficient to accelerate arterial thrombosis. Genetic loss of the β_2AR on alveolar macrophages prevents, and β_2AR agonists augment, the PM-induced release of IL-6 via mitochondrially derived ROS, which

augment adenylyl cyclase/cAMP/CREB-induced transcription. **Conclusions:** These results provide a novel mechanistic paradigm linking activation of the sympathetic nervous system with lung inflammation and an enhanced susceptibility to ischemic cardiovascular events. These results also suggest a mechanism contributing to the unexpected association between the clinical use of β_2AR agonists and mortality in observational studies.

38

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

***Vida Chitsazzadeh*[1], T. Nguyen[4], V. Thomas[2], M. Migden[2], A. Joseph[5], P. Gunaratne[6], C. Coarfa[7], X. Su[3], E. R. Flores[8], K. Y. Tsai[1, 2]**

[1]Department of Immunology; [2]Department of Dermatology; [3]Department of Bioinformatics & Computational Biology; [4]Mohs Micrographic & Dermatologic Surgery Northwest Diagnostic Clinic, Houston, TX; [5]Mohs Micrographic & Dermatologic Surgery Skin and Laser Surgery Associates, Pasadena, TX; [6]Department of Biology and Biochemistry University of Houston, Texas; [7]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; [8]Department of Biochemistry and Molecular Biology, University of Texas MD Anderson Cancer Center Houston, TX

Introduction: Skin cancer is the most common malignancy in humans. Annually, in U.S. there are over 3 million cases with an estimated overall economic impact of $2 billion. Cutaneous Squamous Cell Carcinoma (cSCC) comprises 15-20% of all skin cancers. cSCC has the best-defined progression from a distinct precancerous lesion, the Actinic Keratosis (AK), to invasive cSCC. Destructive therapies for AK treatment must be used repetitively, causing significant morbidity. There is a tremendous need for targeted diagnostics and therapy for AKs, representing an important opportunity for secondary skin cancer prevention. Our knowledge of the molecular and cellular events that lead to the transformation of normal skin (NS) to AK and subsequently to cSCC is very limited, thus representing a fundamental gap in our understanding of this progression. Our long-term goal is to identify important genetic events that determine the progression of clinically normal sun-exposed skin to AK to cSCC, and to target them for the prevention and therapy of cSCC. **Methods:** We have used a novel approach that combines RNA-Seq, miR-Seq, and reference exome sequencing on tissue-specific, matched samples at three stages of tumor development. The power of our study rests in performing inter-lesional analysis on internally controlled lesions on the path to carcinoma. **Results:** Through our initial analysis of 10 sets of matched samples, we have identified miR-181 as a potential molecular target; expression of the entire miR-181 family gradually increases throughout cSCC progression ($P<0.05$). The significant role of miR-181 in tumorigenesis is revealed by various studies that show upregulation of miR-181 in colorectal carcinoma, invasive cervical squamous cell carcinoma, and hepatocarcinoma. We have focused initially on the functional relevance of TGFBR3, a member of the TGF-Beta family. In recent years, TGFBR3 role as a tumor suppressor has been recognized in breast, lung, ovarian, pancreatic and prostate cancer. We hypothesize that upregulation of miR-181 promotes initiation and progression of keratinocyte transformation by targeting TGFBR3. The results of our proposed experiments will provide insights into miR-181 and TGFBR3 role in cell cycle regulation, cellular motility, and impact on epithelial mesenchymal transition (EMT). Better understanding of these mechanisms offers an avenue for therapeutic intervention in both prevention and treatment of cSCC. **Discussion:** Currently, we lack diagnostic predictors of AK progression to cSCC. We propose that miR-181 levels, correlated with TGFBR3 expression, drive disease progression and can be used as a noninvasive biomarker to both predict AK behavior and prognosis in cSCC pathogenesis. Our proposed project is of paramount importance and has a significant translational endpoint that will allow us to identify and ultimately validate biomarkers for cSCC progression and therapeutic targets for cSCC prevention and treatment.

39

Magnetic Resonance and Optical Imaging as a Biomarker for Muscle Damage

***SM Chrzanowski*[1], Lee BA[1], Batra A[2], Vohra R[1], Deng Y[3], Jiang H[3], Vandenborne K[2], Walter GA[1]**

[1]Department of Physiology Functional Genomics, University of Florida, Gainesville, FL; [2]Department of Physical Therapy, University of Florida, Gainesville, FL; [3]Department of Biomedical Engineering, University of Florida, Gainesville, FL

Background: Muscle damage, a common finding in many neuromuscular disorders, can be currently assessed through various mechanisms such as biopsy, functional testing, and Magnetic Resonance Imaging and Spectroscopy (MRI and MRS, respectively). Here, we demonstrate that Optical Imaging (OI), in utilization with an FDA approved fluorescent contrast agent, Indocyanine Green (ICG) can be utilized as a novel minimally invasive modality to detect and quantify muscle damage in an immobilization-reambulation murine model. **Methods:** Single hindlimbs of C57/BL10 mice were cast immobilized in a plantar flexed position for two weeks. Note that because only one limb was casted, the contralateral limb served as the control 'uncasted' limb. Upon removal of the casts, mice were allowed to freely reambulated, and data (MRI, MRS, OI) were captured at various timepoints (0, 1, 2, 3, 5, 7 days) during reambulation. T_2 relaxation times of several muscle groups (Soleus, Gastrocnemius, Tibialis Anterior) were measured using diffusion corrected T_2 Spin Echo Imaging, and separately through a STEAM acquisition Sequence. Upon injection of ICG, OI results were acquired using a 2-D In Vivo Fluorescence Imager (Ex: 745 nm, EM: 820 nm). **Results**: Both MRI and MRS demonstrated an increase of T_2 relaxation times in the Soleus specifically of the casted limbs at two days of reambulation, remaining elevated until the seventh day of reambulation. Neither the Gastrocnemius nor the Tibialis Anterior demonstrated any appreciable change in T_2 relaxation times during the casting or reambulation phase of this experiment. OI, measured by Radiant Efficiency, displayed a parallel trend of a delayed increase in signal during the reambulation period in only the casted limb. **Conclusion:** The immobilization-reambulation protocol used to induce soleus specific damage and repair in a time dependent manner is confirmed by MRI and MRS. For the first time, OI results further demonstrates its potential as a safe and minimally-invasive biomarker to quantify and measure muscle damage.

Poster Abstracts

40

Neutrophil S100A8/A9 Regulates *Aspergillus fumigatus* Growth and Antioxidant Defense Through Zinc Chelation

***Clark HL*, De Jesus TJ, Sun Y, Pearlman E**

Department of Ophthalmology and Visual Sciences, Case Western Reserve University, Cleveland, OH

The filamentous fungus *Aspergillus fumigatus* is a major cause of corneal fungal infection. S100A8 and S100A9 are proteins implicated in a number of immune functions and neutrophil extracellular trap (NET)- associated S100A8/A9 complex (calprotectin) has been shown to directly inhibit growth of *Aspergillus* species in vitro. We sought to identify a role for these proteins in a mouse model of *A. fumigatus* keratitis. We injected *A. fumigatus* spores intrastromally in either C57BL/6 or S100A9* knock-out mice. At 48 hours we harvested eyes and cultured homogenates to calculate CFU. The S100A9-/- mice had a significantly higher CFU than wild-type mice. Despite a reported role for S100A8 and S100A9 in neutrophil recruitment, we found no difference in neutrophil infiltration between wild-type and S100A9-/- mice. To evaluate neutrophil fungal killing in vitro, we harvested peritoneal neutrophils from C57BL/6 or S100A9-/- mice and incubated them with *A. fumigatus* hyphae for 16 hrs. The S100A9-/- neutrophils demonstrated inferior fungal killing compared to wild-type neutrophils. Fungal killing by wild-type neutrophils was impaired by the addition of excess Zn^{2+}. We also found that hyphae have increased sensitivity to oxidative stress in a low Zn^{2+} environment, suggesting that zinc chelation synergizes with neutrophil reactive oxygen species mediated fungal killing. Finally, we found that anti-malarial drug atovaquone inhibits fungal growth and reduces the labile zinc content of hyphae. Taken together, these data indicate that S100A8/A9 has a non-redundant role in fungal keratitis. S100A8/A9 chelates Zn^{2+} which is necessary for fungal growth and survival in the presence of oxidative stress. We identified atovaquone as a zinc targeting drug that has potential as a novel anti-fungal therapy.

**S100A9 knock-out mice also have extremely low levels of S100A8 in myeloid cells, making these mice functional double knock-outs*

41

Assessment of Bladder Cancer Cell Line Heterogeneity and Therapeutic Sensitivity

***C. J. Claunch-Rabe*, T. G. Levin, T. Koppie, J. Korkola, J. W. Gray**

Oregon Health and Science University, Portland, OR

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

42

Dnmt3a is Required for Aberrant Self-Renewal Driven by *PML-RARA*

***CB Cole*[*†], AM Verdoni[*], DH Spencer[*], TJ Ley[*]**

[*]Department of Medicine and [†]MD-PhD Program, Washington University School of Medicine, St. Louis, MO

We previously identified recurrent loss of function mutations in the DNA methyltransferase *DNMT3A* in patients with acute myeloid leukemia (AML), which are associated with a poor prognosis. DNMT3A catalyzes the *de novo* methylation of specific DNA sequences during differentiation. Loss of *Dnmt3a* in hematopoietic stem cells impairs their ability to differentiate into committed progenitors. Importantly, *DNMT3A* mutations are mutually exclusive of the favorable prognosis t(15;17) translocation, which creates the *PML-RARA* fusion gene. PML-RARA has been shown to interact with DNMT3A *in vitro*, and to require DNMT3A to induce methylation and transcriptional silencing of a subset of specific target genes. These findings suggest that *PML-RARA* may require functional *DNMT3A* to initiate leukemia. To investigate the role of *Dnmt3a* in leukemia initiated by *PML-RARA*, we utilized a well-characterized transgenic mouse model in which expression of *PML-RARA* is driven in early hematopoietic stem/progenitor cells by the mouse *Cathepsin G* locus (*Ctsg-PML-RARA*). These mice spontaneously develop acute promyelocytic leukemia (APL) with high penetrance and long latency, and also exhibit a preleukemic phenotype marked by increased contribution to peripheral blood cells in competitive transplant assays. In addition, myeloid progenitor cells derived from these mice have the ability to serially replate in methylcellulose cultures,

demonstrating aberrant self-renewal *ex vivo*. We generated *Ctsg-PML-RARA* mice lacking *Dnmt3a* (*PML-RARA+/- x Dnmt3a-/-*). Loss of *Dnmt3a* completely abrogated the *ex vivo* replating ability of *PML-RARA* bone marrow. Competitive repopulation experiments with *PML-RARA+/-* x *Dnmt3a-/-* marrow revealed a decreased contribution to peripheral blood cells as well as spleen and bone marrow cells at 12 weeks, relative to *PML-RARA+/-* or WT control animals. Characterization of the effect of *Dnmt3a* loss on leukemia incidence and phenotype is being studied in an ongoing tumor watch, but preliminary data demonstrates a statistically significant decrease in APL penetrance at 1 year. In summary, we present data suggesting that *Dnmt3a* is required for leukemogenesis induced by *PML-RARA*. These findings may explain the clinical observation that *DNMT3A* mutations are exclusive of *PML-RARA*.

43

An Extra-Catalytic Function of the Tyrosine Phosphatase CD45 in Lymphocytes

***S.E. Coughlin*[*‡], W.C. Raschke[†], A. Weiss[*‡], J. Zikherman[*]**
[*]Department of Medicine, UCSF, San Francisco, CA; [†]Virogenics Inc, San Diego, CA ‡HHMI

CD45 is a receptor-like protein tyrosine phosphatase, highly expressed in all nucleated hematopoietic cells, that serves as a positive regulator of antigen receptor signaling by dephosphorylating the inhibitory tyrosine of Src family kinases. CD45 consists of a large, heavily glycosylated extracellular (EC) domain, a single transmembrane (TM) domain, and a cytoplasmic segment that contains tandem protein tyrosine phosphatase domains. While CD45 phosphatase activity and its substrates are relatively well described, the function of the extracellular domain remains poorly understood; no convincing ligand has been identified. Importantly, alternative splicing of CD45 yields isoforms that differ in their extracellular domains, and expression of these isoforms is tightly regulated in a cell type- and activation state-dependent manner. The highly regulated isoforms suggests that the extracellular domain has an important yet-to-be-defined function. We aim to determine if CD45 exhibits any function independent of its protein tyrosine phosphatase activity in antigen receptor signaling. To do so we utilize two transgenic mouse lines lacking phosphatase activity. The first contains a cysteine to serine (CS) point mutation in the D1 phosphatase domain that renders CD45 phosphatase inactive. In the second line, the cytoplasmic domain is deleted entirely (DelC). The CS and DelC were superimposed upon a background of varying levels of functional CD45 using a mouse allelic series. We show that expression level of the EC and TM domains of CD45 can alter cellular responses independent of phosphatase function. We observe no effect of CS or DelC on T cell development, signaling, or activation. By contrast, catalytically inactive CD45 has numerous effects on B cells. CS and DelC transgene expression is associated with decreased surface IgM and a reduced marginal zone B cell population. Signaling studies reveal increased ERK phosphorylation in response to B cell receptor (BCR) stimulation in the presence of CS and DelC transgenes, whereas we surprisingly observe a decrease in intracellular basal calcium, and B cell activation is impaired. Given these findings we hypothesize that the CD45 CS and DelC proteins modulate BCR signaling, perhaps via association with co-receptor(s). Similar CS and DelC phenotypes in CD45 null B cells argues against a dominant negative effect on endogenous CD45 as an alternative explanation. In sum, we find evidence of extracatalytic function of CD45 in antigen receptor signaling in B cells, but not in T cells.

44

A Mouse Model for Analysis of Modifications to Increase the Safety of the Live Attenuated Influenza Vaccine

***A. Cox,* S. Baker, L. Martínez-Sobrido, S. Dewhurst**
University of Rochester, NY

Influenza A virus (IAV) is a respiratory pathogen that infects through the upper airway and creates pathogenesis through replication in the lower airway. The temperature gradient between these two areas led to the development of the live attenuated influenza vaccine (LAIV) that can replicate in the cooler upper respiratory tract and trigger a protective immune response, while its replication is limited in the lower respiratory tract, allowing for safe administration. This temperature sensitivity (ts) is imparted by 5 mutations within the viral replicative machinery: namely PB2 N265S; PB1 K391E, D581G and A661T; NP D34G. Though this vaccine has an overall acceptable safety profile, it is not licensed in children under two due to concerns of elevated hospitalizations due to wheezing. For this reason it is also not licensed in asthmatics or the immunocompromised. However, this vaccine showed greater efficacy than its inactivated counterpart in initial clinical trials. Therefore modifications to this vaccine that maintain efficacy while increasing safety are desirable. However, to date this has been hampered by an inadequate model for safety, as LAIV shows little to no pathogenicity in mice or ferrets. Here we implement a previously described PR8 virus containing the 5 ts loci of LAIV and show that it has greatly reduced pathogenicity in mice, but importantly still retains lethality at easily administrable doses (10^5 FFU). This attenuated virus also maintains protective efficacy against homologous and heterologous IAV challenge. This platform can be used to evaluate the safety and protective efficacy of modifications to the existing LAIV.

46

A Novel Phosphorylation Site Influences Multiple Regulatory Functions of Papillomavirus E2 Protein

***SP Culleton*[*], EJ Androphy[*]**
[*]Indiana University School of Medicine, Indianapolis, IN

Human papillomavirus (HPV) remains the most common sexually transmitted disease in the United States. Since the HPV vaccine only prevents infection, new strategies are needed to combat the virus in those already infected. One potential treatment target is the amplification of viral genomes upon terminal differentiation of their keratinocyte hosts, a switch that allows for efficient spread of the virus but whose mechanisms remain poorly understood. Our lab has addressed this issue by examining the viral protein E2 – the master replication and transcription regulator of HPV – and the cellular factors that influence its behavior. Our current study focuses on post-translational modification (PTM) of E2 from bovine papillomavirus type 1 (BPV1), a model system for HPV. We discovered a novel phosphorylation site in E2, tyrosine

102, after purifying the protein from transfected C33A cells (an HPV-negative cervical cancer cell line) and subjecting samples to linear ion trap mass spectrometry. This PTM appeared at the highest confidence level out of all PTMs detected, including two previously published phosphorylation sites at serines 298 and 301. To explore the significance of P-Y102 further, we used site-directed mutagenesis to create phospho-deficient and phospho-mimetic point mutants (Y102F and Y102E, respectively) and used transient transfections to study the capacity of these mutants for transactivation, replication, and interaction with other factors relevant to these processes. While Y102F exhibited transcription and replication capabilities comparable to wild type in a luciferase-based reporter model, the phospho-mimetic mutation abolished these functions. In immunoprecipitation experiments, wild-type E2 and Y102F precipitated known E2 binding partners, including cellular transcriptional coactivators and the viral replication factor E1; however, despite protein levels comparable to wild type and Y102F, Y102E failed to bind all factors tested except for a fragment of the chromatin remodeling protein Brd4. We hypothesize that phosphorylation at Y102E occurs during viral maintenance to inhibit E2 activity and that its removal allows E2 to initiate amplification of the genome and virion production. Identifying the kinase controlling this modification will provide a deeper understanding of the host cell's influence on papillomavirus life cycle.

48

Targeting FLT3 Induces Lethal Autophagy in Acute Myeloid Leukemia via CerS1 Mediated C18-Ceramide Generation

Dany M [1,2], **Ogretmen B**[1,2]

[1]*Department of Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston, SC;* [2]*Hollings Cancer Center, Medical University of South Carolina, Charleston, SC*

Mutations in fms-like tyrosine kinase 3 (FLT3) are common in Acute Myeloid Leukemia (AML) and confer a worse prognosis. Thus, FLT3 inhibitors are promising therapeutic agents for AML treatment. Ceramide, the bioactive sphingolipid, mediates cell death in response to various chemotherapeutic agents via apoptosis, necroptosis, mitophagy, or autophagy. The regulation of cell death-related sphingolipid signaling in FLT3-associated AML is currently unknown. This study investigates the regulation and function of Ceramide Synthases (CerS1-6) in AML oncogenesis and its susceptibility to FLT3 inhibitors. Our studies show that drug resistance in AML to FLT3 inhibitors (Sorafenib, Quizartinib, Crenolanib) is partly associated with decreased levels of C18-ceramide generation due to downregulation of ceramide synthase 1 (CerS1). We showed that CerS1 expression is suppressed in AML cells expressing FLT3-ITD mutation compared with FLT3 negative AML cells and normal CD34+ hematopoietic stem cells. Treating FLT3 positive cells with FLT3 inhibitor Crenolanib selectively induced the expression of CerS1 and the generation of C18-ceramide after 24 h and 48 h. Interestingly, siRNA mediated knockdown of CerS1 expression partially protected AML cells from Crenolanib-mediated cell death. Mechanistically, Crenolanib/CerS1/C18-ceramide axis seemed to induce lethal autophagy, as determined by increased LC3B lipidation. Knockdown of LC3B using siRNAs partially protected FLT3-ITD AML cells against Crenolanib-induced death. Importantly, no PARP-1 cleavage was detected and caspase 3 inhibitor failed to protect the cells from drug induced cell death ruling out apoptosis. In summary, our novel data suggest that FLT3 inhibitors reactivate CerS1/C18-ceramide axis leading to induction of autophagy and cell death. This suggests that lipid metabolism could be a potential target for treating AML and reducing resistance in patients receiving FLT3 inhibitors.

49

G-CSF Induces Alterations in the Bone Marrow Microenvironment That Suppress B Lymphopoiesis

RB Day*†*, **A Greenbaum****†*, **and DC Link***

**Department of Medicine;* †*MD-PhD program, Washington University in St. Louis, St. Louis, MO*

The production of hematopoietic cells in the bone marrow is tightly and dynamically regulated in response to environmental stimuli. In response to infection, the bone marrow increases granulopoiesis at the expense of lymphopoiesis. The mechanisms mediating this shift are poorly defined. Because the bone marrow microenvironment protects leukemic cells, better understanding the factors inducing a lymphoid-toxic microenvironment may provide novel strategies for sensitizing lymphoid malignancies to chemotherapy. Here we report that exogenously-administered granulocyte colony-stimulating factor (G-CSF), a key regulator of granulopoiesis induced during infection, is associated with marked loss of B cells in murine bone marrow. After 5 days of G-CSF treatment, total B cells in the bone marrow are reduced 8.1 ± 0.9-fold. All committed stages of bone marrow B lymphopoiesis from the B-committed lymphoid progenitor to mature naïve B cells are suppressed but the number of the upstream common lymphoid progenitor (CLP) is unchanged. The loss of bone marrow B cells is predominately explained by mobilization and apoptosis. G-CSF receptor-deficient bone marrow chimeras show that G-CSF acts in a non-cell intrinsic fashion to suppress B lymphopoiesis. Consistent with this observation, G-CSF decreases the expression of important B-supportive factors in the bone marrow microenvironment, including CXCL12, kit ligand, flt3 ligand, interleukin-6, interleukin-7, IGF-1, and BAFF. We also observe decreased CXCL12 production from two stromal cell populations important in B cell development, osteoblasts and CXCL12-abundant reticular (CAR) cells. To assess the role of CXCL12 production by these cell types to B lymphopoiesis, we generated *Cxcl12*^{flox} mice and crossed them with mice expressing tissue-specific *Cre-recombinase* transgenes. Deletion of *Cxcl12* using *Oc-Cre* (targeting mature osteoblasts) results in an isolated loss of mature naïve B cells in the bone marrow due to a failure to home to the bone marrow following peripheral maturation. Deletion of *Cxcl12* using *Osx-Cre* (targeting CAR cells) results in an earlier loss of bone marrow B cells beginning with pre-pro-B cells. Deletion of *Cxcl12* using *Prx1-Cre* (targeting mesenchymal progenitors) results in severe suppression of B lymphopoiesis, including a loss of CLP, the upstream progenitor of B, T, and NK cells. Interestingly, treatment of *Prx1-Cre Cxcl12*$^{flox/-}$ mice with G-CSF results in additional B cell loss, indicating that deletion of *Cxcl12* in mesenchymal stromal cells is not sufficient to fully recapitulate G-CSF-induced B cell suppression.

Poster Abstracts

50

4-HNE Post-Translational Modification of TRPV1: Implications for Diabetic Cardiomyopathy

***Daniel DelloStritto*†, John G. Kmetz†, Brittany Klarich†, Yeong-Renn Chen†, Werner Geldenhuys† and Ian N. Bratz†**
†*Department of Integrative Medical Sciences, Northeast Ohio Medical University (NEOMED), Rootstown, OH*

Previously our lab has shown TRPV1 -dependent regulation of myocardial blood flow (MBF) is disrupted in a db/db mouse model of diabetic cardiomyopathy (DCM). A crucial mechanism behind pathologies seen in diabetes is the imbalance in oxidative stress (OS) leading to increased lipid peroxidation (LPO). The LPO product 4-hydroxynonenal (4-HNE) is known to increase oxidative post translational modification (PTM) resulting in altered protein function. Investigation into the implications of this oxidative PTM on TRPV1 has yet to be examined. Accordingly, we hypothesized that 4-HNE PTM of TRPV1 results in decreased functional expression leading to perfusion impairments in DCM. Initial experiments were performed to evaluate 4-HNE modification of TRPV1. Pull down experiments on HEK cells constitutively expressing TRPV1, treated with H_2O_2 (to simulate OS) and 4-HNE, resulted in increased 4-HNE modified TRPV1 compared to control with a concomitant decrease in TRPV1 expression. Similar findings were demonstrated in hearts from diabetic animals and controls treated with H_2O_2 and 4-HNE. Computer generated modeling identified numerous possible residues capable of undergoing 4-HNE modification of which several were confirmed using mass spectroscopy. TRPV1 functional analysis using path-clamp electrophysiology revealed blunted capsaicin-mediated currents in the presence of 4-HNE and/or prolonged H_2O_2 treatment. Similarly, 4-HNE decreased capsaicin mediated increases in MBF and relaxation in isolated coronary microvessels. These data suggest that increased OS in a diabetic state leads to decreased TRPV1 functional expression resulting in perfusion impairments seen in DCM.

51

TSA Blocks Advanced Glycation End-Product-Induced RPE Barrier Breakdown in a Model of Diabetic Eye Disease

***Danielle Desjardins,* Mohammad Dahrouj, Yueying Liu, Craig Crosson, Zsolt Ablonczy**
Medical University of South Carolina, Charleston, SC

Purpose: Diabetic retinopathy is best known for its microvascular complications; however, there is mounting evidence that the retinal pigment epithelium (RPE) is also involved. Advanced glycation end products (AGE), which are elevated in diabetes, have recently been shown to contribute to RPE barrier dysfunction. Barrier function is known to be altered in the pathogenesis of several CNS disorders which has been linked to alterations in protein acetylation and histone deacetylase (HDAC) activity. Here, we provide new evidence that the administration of Trichostatin A (TSA), a pan-HDAC inhibitor, suppresses the RPE barrier breakdown induced by AGE *in vitro* and *in vivo*. **Methods**: Monolayers of ARPE-19 or human fetal RPE (hfRPE) cells were treated (100µg/mL) with bovine albumin (BSA) or glycated bovine albumin (gBSA) in the presence of vehicle (DMSO, 0.001%) or TSA (100nM). Transepithelial resistance (TER) was measured at 0, 4, and 6h post administration. Dutch-belted rabbits were injected (20µL) intravitreally with albumin or gBSA (1mg) in the presence of vehicle (DMSO, 0.1%) or TSA (3.0µg). Subretinal blebs (5µL PBS) were injected 2 days later and observed via optical coherence tomography. Volumes and rates of resorption were calculated by following blebs for 1h. **Results**: Baseline TER in ARPE-19 and hfRPE monolayers was 57±4.4 and 291±62 milliohms, respectively. Administration of gBSA resulted in a 13% ($p<0.001$) and 25% ($p<0.001$) reduction in ARPE19 and hfRPE TER, respectively. In ARPE-19 and hfRPE monolayers co-treated with gBSA and TSA, decrease in TER was blocked. The administration of BSA or TSA alone did not significantly alter TER in either preparation. Rabbit eyes treated with BSA had a resorption rate of 11.01±2.04 $ul*cm^{-2}*hr^{-1}$ which was reduced to 2.79±0.83 $ul*cm^{-2}*hr^{-1}$ with gBSA ($p<0.05$, n=4). The decrease in the rate of fluid resorption induced by gBSA was blocked by co-treatment with TSA (n=4). **Conclusions**: The administration of TSA prevented RPE barrier dysfunction induced by AGE- both *in vitro* and *in vivo*. These data provide evidence that HDAC activity is important in the development of AGE-induced RPE dysfunction. The suppression of AGE-induced changes in RPE function *in vivo* by TSA supports the idea that HDAC inhibitors may be efficacious in the treatment of diabetic macular edema.

52

Evaluation of Cellular Proliferation in Vein Grafts

***L. Dumeny,* Y. Hu, A. Cuenca, K. O'Malley, Z. Jiang, S. Berceli**
Malcom Randall Veterans Administration Medical Center, Gainesville, FL; University of Florida, Gainesville, FL

Vein grafts are a form of treatment for vascular diseases, but often only provide short-term improvement due to the possibility of grafts failure. The primary mechanism of failure is due to intimal hyperplasia and inward remodeling of the graft. The placement of a vein into an arterial circulation leads to changes in shear stress due to the graft being placed into a different circulation or shear environment. Thus, it is hypothesized that the shearing force across the endothelium leads to proliferation of cells within the vessel and causes biochemical and morphological changes to the graft. In this study, we sought to find a correlation between the rate and timing of cell proliferation with intimal hyperplasia development. In order to find a correlation, vein graft were generated, harvested, and stained for bromodeoxyuridine (BrdU) and Ki-67. These immunohistochemical stains were used to assess cell proliferation at 2 hours, 1, 3, 7, 14, and 28 day(s) after implantation in rabbits. Positive cells are visibly stained and were counted to determine the proliferation rate. We found that while the low and high flow vein grafts had a similar adaptation in proliferation and intimal growth, the 7 day time point showed the most variation and difference between the two flows. We conclude that the 7 day time point is significant for IH development and vein graft adaptation. These data will be used as part of a model to understand the biological and physical effects of the vein graft and the process of vascular adaption from the injury.

Poster Abstracts

53

Induction of Pancreatic Tumor Formation by Oncogenic Kras Requires PI3K p110α

ES Carpenter,* **CY Wu, KK Takeuchi, H Bien, RZ Lin, HC Crawford**

**Department of Physiology and Biophysics, Stony Brook University, Stony Brook, NY*

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer-related death worldwide, with a five year survival rate of 6%. Since current treatment strategies have little impact on disease progression, understanding the molecular mechanisms responsible for PDAC to find new therapeutic targets is of paramount importance. Almost all PDAs are caused by mutations in the Kras gene. While pharmacological inhibition of Kras has so far proven unsuccessful, Kras does activate several signaling 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-KrasG12D (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 in the mouse pancreas completely abrogated the development of pancreatic tumors in KC mice without causing detectable harmful effects, whereas the p110β subunit was dispensable. Specifically, we found that acinar cells deficient in p110α were capable of metaplastic transformation in vitro, but were unable to maintain stable metaplasia in vivo. When challenged with the pancreatic secretagogue cerulean, p110α-null pancreata were protected against active actin-cytoskeleton arrangement seen in KC pancreata, indicating that p110α is required for the actin-cytoskeleton rearrangement seen in metaplasia. Upon exploring signaling effectors downstream of PI3K, we found that the KC pancreas exhibited increased 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. To further explore the mechanism by which p110α signals to Rac1, we found that Vav1, a Rac1 GEF that has been implicated in pancreatic cancer, was overexpressed in KC acinar cells as compared to p110α-null KC acinar cells. Furthermore, Vav1 was found to be transcriptionally upregulated following expression of oncogenic Kras and transcriptionally downregulated following genetic silencing of p110α. In summary, we found that Kras requires and activates the p110α-Rac1 pathway to induce pancreatic tumors. 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.

54

Origin and Lifecycle of Cardiac Macrophage Subpopulations in Steady State and Inflammation

***Slava Epelman**[1],* **KJ Lavine[1], GJ Randolph[1], D Sojka[1], W Yokoyama[1], J Carrero[1], B Calderon[1], ER Unanue[1], M Colonna[1], EC Forsberg[2], AE Beaudin[2]**

[1]Washington University School of Medicine, St. Louis, MO; [2]UCSC, Santa Cruz, CA

Background: Cardiovascular disease is the leading cause of death in industrialized nations and cardiac macrophages (MFs) are critically involved in the response to tissue injury. Unfortunately, therapeutic progress has been limited due to an inability to understand and resolve very heterogeneous populations. Using genetic fate mapping, adoptive transplant of fetal liver stem cells, parabiosis and transcriptional profiling, we have for the first time defined in detail the origin, trafficking and function of distinct resident cardiac MFs subsets during steady state and inflammation. **Results:** Genetic fate mapping revealed that yolk-sac and fetal monocyte progenitors gave rise to the majority of adult resident cardiac MFs. CCR2 expression distinguished monocyte-derived from embryonic-derived cardiac MFs. Importantly, significant persistence of E8.5 labeled primitive (yolk sac) MFs into adulthood was restricted to brain microglia, and subsets of cardiac and liver MFs. Following MF depletion, a limited window opened, where embryonically established resident cardiac MFs were replenished through both self-renewal and replacement by long-lived adult blood Ly6c^{Hi} monocyte-derived MFs. After reconstitution of the resident MF compartment was complete, the autonomy between tissue and blood was re-established. This process of embryonic MF replacement by blood monocytes also extended to resident MF populations in other organs, suggesting a generalizable mechanism. During cardiac inflammation, Ly6c^{Hi} monocytes drove the expansion of multiple MF populations, while resident MFs expanded through proliferation. Transcriptional and functional analyses of resident cardiac MFs revealed that adult monocyte-derived MFs coordinate cardiac inflammation, while playing redundant but lesser roles in antigen presentation and uptake of apoptotic cardiomyocytes. **Conclusions:** These data define a detailed landscape of multiple cardiac MF subsets in resting and stressed conditions, with different ontological origins, strategies to regulate compartment size and functions. Moreover our data create an ontological framework to target pathways pathological pathways within specific MF subsets, while leaving untouched protective MF subsets.

55

Oxidative Stress Variation in Patients with Heart Defects

***Rebecca Fabian**[1],* **Mark W. Russell[1,2], Sarah Geisler[2]**

[1]UROP, University of Michigan, Ann Arbor, Michigan; [2]Department of Pediatric Cardiology

A deletion of chromosome 2q13 was found in patients with cardiac defects and craniofacial abnormalities, most notably Double-Outlet Right Ventricle (DORV), pulmonary atresia, Ventricular septal defect, and tetralogy of Fallot. An overlap of six missing genes was discovered and narrowed down to four major genes that played

a role in the defects. They are MERTK, FBLN87, TMEM87B, and ZC3H8 (the other two minor genes were ZC3H6 and ANAPC1). The aim of this study is to use zebrafish as an animal model to look at the different genes, knock out combinations of genes, and observe the effects. With the Morpholino injections that block translation or inhibit splicing, cardiac abnormalities arose when FBLN7A, FBLN7B, and TMEM87B were inhibited, leading to the belief that FBLN7 and TMEM87B seem to be important genes involved with these defects. Further research will be conducted to narrow down the genes causing defects by examining DNA from patients born in Michigan between 2005 and 2010. The project findings will help the entire community of cardiology. If it is proved that there is a link to specific genes in heart defects, then it will be easier to understand the defect, easier to treat the defect effectively, and patient viability will increase.

56
Mitochondrial Haplogroups in Pulmonary Arterial Hypertension

Samar Farha, **Suzy A. Comhair, Micheala A. Aldred, Serpil C. Erzurum**
Cleveland Clinic, Cleveland, OH

Introduction: There is growing evidence that pulmonary artery endothelial cells in pulmonary arterial hypertension (PAH) exhibit a switch in metabolic phenotype characterized by low numbers of mitochondria, decreased oxygen consumption, higher glycolytic rate, apoptosis-resistance, and increased cell proliferation. The metabolic changes are also seen in the cardiac myocytes in PAH. Data suggest that mitochondrial dysfunction and altered cellular metabolism contribute to the pathogenesis of PAH. Mitochondrial (mt) DNA encodes for central genes of the mitochondrial energy-generating process oxidative phosphorylation. Accumulation of random mtDNA mutations produced clusters of related mtDNA haplotypes known as haplogroups. Mitochondrial haplogroups are associated with differences in metabolic rates and energy expenditure. Here, we hypothesize that specific mitochondrial haplogroups, which influence cellular bioenergetics are associated with the glycolytic shift in PAH. To test this hypothesis, we determine the distribution of mitochondrial haplogroups in PAH and control groups. **Methods:** DNA from PAH patients and controls were analyzed. Using taqman PCR, we genotyped unique single-nucleotide polymorphism (SNP) that identify specific haplogroups (H, UK, K, L, M, T, V, J). We identified mitochondrial haplogroups in 210 samples (121 PAH patients and 89 controls) out of 304 samples. **Results:** The race distribution of our genotyped population was as follow with the most common races being Caucasian and African American: 172 Caucasian (PAH 102, controls 70), 29 African American (PAH 11, controls 18), 5 Asian (PAH 5, controls 0) and 4 Hispanic (PAH 3, controls 1). The analysis was limited to Caucasian and African American as other races were under represented. As expected, mitochondrial haplogroups were dependent of race (Chi square $p < 0.001$). Association of mitochondrial haplogroups to disease state in African American races was not significant (Chi square $p > 0.1$). In Caucasian, there was a trend for decreased haplogroup H in PAH (37%) compared to controls (51%) (Chi square $p = 0.067$). In parallel, haplogroup T was more frequent in PAH (23%) compared to controls (13%) though not statistically significant (Chi square $p = 0.1$). **Conclusions:** In our cohort of PAH patients and controls and controlling for racial effect on mitochondrial haplogroups, we found a trend for decreased frequency of PAH in Caucasians of H haplogroup. These findings suggest a possible role for mtDNA variations in the mechanisms of mitochondria dysfunction in PAH.
Supported by RO1 HL60917 and AHA 0835146N

58
Incidence and Risk Factors for Opioid Induced Respiratory Depression and Sedation at an Academic Medical Center

ET Fiedler, **S Reingold**
Loyola University Stritch School of Medicine

Background: Opioids have the potential to cause respiratory depression (RD), which can result in prolonged hospital stays or life threatening conditions. TJC recently released an Alert highlighting the need to improve the safety of opioids used in pain management, specifically emphasizing the dangers of RD. ADEs resulting from RD can occur in young healthy patients, but patients with sleep apnea, snoring, morbidly obese patients, pediatric patients, geriatric patients, patients receiving other sedatives, smokers, patients with pre-operative opioid tolerance and surgical incisions that may impair breathing are all at a higher risk. Medical errors also contribute to opioid ADEs. Retrospective studies consistently find that moderate to severe opioid induced RD occurs in 0.5-2.0% of inpatients receiving opioids, while one continuous monitoring study found the actual rate to be 12%. **Methods:** The rate of adverse events associated with opioids at Loyola University Medical Center (LUMC) is not known. Instances of acute RD and sedation from opioid toxicity are treated using naloxone, an opioid receptor antagonist. A query of the Epic database, LUMC's electronic medical record, revealed 303 cases of inpatient visits in which an opioid and naloxone were administered to a patient during 2012. We are in the process of conducting a retrospective review of these records to determine the risk factors and incidence of opioid induced RD or sedation resulting in naloxone rescue at LUMC. **Results:** To date, 87 patient records have been analyzed. Of these, 22 patients had experienced episodes of RD or sedation. We have made several important preliminary findings. First, 19 out of 22 patients with RD or sedation received at least two different opioids, and frequently were receiving three opioids administered via three different routes. These complicated dosing schedules expose patients to greater potential for medication errors. Second, 15 out 22 of patients experiencing RD or sedation had elevated creatinine levels. These findings suggest that patients with renal disease are particularly at risk for serious opioid related ADEs. Finally, patients with surgical sites in the thorax and abdomen are well represented in this sample. Suggesting that the depressive effects of opioids on respiration are compounded by surgical trauma to the muscles of respiration. **Conclusions:** Our findings suggest that LUMC patients receiving a complicated regimen of opioids, or who have renal disease, or patients who have undergone thoracic or abdominal surgery would benefit from additional respiratory monitoring while receiving opioid analgesia.

Poster Abstracts

59

trkB Signaling Mediates Neuroprotective and Behavioral Effects of Long-Term, High-Frequency Subthalamic Nucleus Deep Brain Stimulation

DL Fischer, **NK Polinski, CJ Kemp, A Cole-Strauss, JW Lipton, K Steece-Collier, KL Paumier, TJ Collier and CE Sortwell**

Department of Translational Science & Molecular Medicine, Michigan State University, Grand Rapids, MI

High-frequency deep brain stimulation (DBS) of the subthalamic nucleus (STN) is the most common neurosurgical treatment for the alleviation of Parkinson's disease (PD) motor symptoms. Beyond symptomatic efficacy, our laboratory and others have demonstrated that high-frequency DBS of the subthalamic nucleus (STN) provides neuroprotection for dopaminergic neurons of the substantia nigra pars compacta (SNc) and increases brain-derived neurotrophic factor (BDNF) in the SNc in the 6-hydroxydopamine (6-OHDA) rat model of PD and in the striatum of unlesioned rats (Spieles-Engemann et al., Neurobiol Dis, 2010; Spieles-Engemann et al., J Parkinsons Dis, 2011). However, whether BDNF participates in either the neuroprotection or behavioral effects or if BDNF is an epiphenomenon remains unknown. In the present study we investigated the impact of ANA-12, an antagonist for the trkB BDNF receptor in the brain (Cazorla et al., J Clin Invest, 2011), using our STN DBS paradigm. We conducted long-term, STN DBS in male, Sprague Dawley rats that received unilateral, intrastriatal 6-OHDA. Stimulation was initiated ten days following 6-OHDA (~25% loss of SNc DA neurons) with rats randomly assigned to receive ACTIVE or INACTIVE continuous stimulation for eighteen days. Within each cadre, rats were randomized to receive ANA-12 (0.5 mg/kg) or vehicle twice per day for the remainder of the study. Forelimb use asymmetry measured via the cylinder task was used to evaluate functional efficacy of STN DBS. Electrode placement in the STN was verified post mortem. Tyrosine hydroxylase immunoreactive (THir) neurons of the SNc were quantified using unbiased stereology. Preliminary data suggest that ANA-12 treatment: (1) abolishes the neuroprotective effect of ACTIVE STN DBS and (2) attenuates the functional effects of ACTIVE STN DBS. These results highlight the importance of BDNF-trkB signaling in the functional and morphological effects of STN DBS.

Supported by Spectrum Health (DLF) and the Morris K. Udall Center of Excellence for Parkinson's Disease Research at Michigan State University (TJC).

60

Characterizing the Properties and Consequences of Alpha-Synuclein Aggregation

W Flavin, **D Freeman, E Campbell**

Loyola University Chicago, Maywood, IL

Key to the development of new treatments aimed at arresting Parkinson's disease (PD) progression will be understanding the mechanisms by which endogenous alpha-synuclein (a-syn) becomes pathologic, how misfolded monomeric a-syn aggregates into oligomers or fibrils, and how these higher-order species induce dysfunction and ultimately propagate their deadly spread despite innate defense mechanisms. Based on its ability to form an amphipathic a-helix and induce membrane curvature, our lab has demonstrated that a-syn gains access to the cytosol by disrupting the lysosomal membrane after endocytosis. This rupture leads to detrimental consequences such as the generation of mitochondrial reactive oxygen species (ROS) and the activation of the inflammasome. Many studies investigating the most pathological form of a-syn have led to a shift of focus away from large proteinaceous inclusions known as Lewy bodies and toward smaller oligomeric species of a-syn. These oligomeric species, capable of entering a cell via endocytosis, would then be directly responsible for lysosomal and mitochondrial dysfunction in our disease model. Because vesicle rupture would provide a key link between the aggregation of a-syn and the neuronal dysfunction and death that occurs in PD, and could explain the spread of a-syn pathology from cell to cell, our lab is interested in examining the various abilities of different forms of a-syn to induce vesicle rupture and ROS production. In order to more fully understand how certain disease-associated mutations or modifications of a-syn play a role in its ability to rupture lysosomal membranes and induce ROS, we are beginning to characterize the properties of a-syn aggregation over time, so as to correlate more severe pathology with certain species of a-syn and the aggregation properties inherent to them. We have begun to examine the relative oligomerization of WT, WT p~S129, A30P, E46K, and A53T a-syn using denaturing and non-denaturing gel analysis, K114 staining, and fluorescence intensity distribution analysis. This type of analysis will allow us to generate aggregates with similar fibrillar content, facilitating a direct comparison of the ability of each species to induce vesicle rupture after standardizing for oligomeric size. In parallel, we are also investigating the ability of these species of a-syn to induce vesicle rupture in N27 and SH-SY5Y cells stably expressing mCherry-Galectin3 as previously described, as well as measuring the ability of each form of a-syn to induce cathepsin-dependent ROS in these cell lines. Our studies are ongoing, but we expect informative results that will aid in the understanding of PD pathogenesis.

Poster Abstracts

61

The Role of Alpha-T-Catenin in the Pathogenesis of Toluene Diisocyanate-Induced Occupational Asthma

***S. Sai Folmsbee*[1,2] and Cara J. Gottardi[2]**
[1]Driscoll Graduate Program in the Life Sciences, [2]Division of Pulmonary and Critical Care Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL

Rationale: 10-25% of adult onset asthma is occupation-induced, a subtype defined by the exposure of environmental irritants to susceptible individuals. Recently, a genome-wide association study, with subsequent independent replication, has shown that alpha-T-catenin mutations, which were associated with its reduced expression, correlate with the incidence and severity of toluene diisocyanate (TDI) induced asthma, the most common identified cause of occupational asthma. Alpha-T-catenin is normally found in testis and heart, and it is a component of cadherin-based intercellular junctions. The alpha-T-catenin-expressing lung cell type and its contribution to the pathogenesis of asthma are unknown. **Methods:** Alpha-T-catenin knockout (KO) mice, which are viable and fertile, were obtained from Dr. Frans Van Roy. Mouse lungs were processed for immunofluorescence (IF) staining using standard fixation/paraffin-embedding procedures. Pulmonary vein muscle wall thickness was quantified by dividing the area bounded by the muscle wall by the lumen area from tissue section images. RNA isolated from mouse lung and heart tissue was analyzed by qRT-PCR using the SYBR Green system. **Results:** qRT-PCR analysis of alpha-T-catenin WT and KO mice demonstrates that alpha-T-catenin mRNA is present in lung at a level that is 20 times less abundant than heart, indicating its presence in a rare cell type. No statistically significant difference in alpha-T-catenin mRNA expression was observed in the lungs of asthmatic mice vs. sham controls using a dust-mite extract asthma model. Alpha-T-catenin was not detected in vascular smooth muscle, bronchial smooth muscle, or airway epithelium by western blot or IF analysis. Alpha-T-catenin was clearly present in cardiomyocytes surrounding pulmonary veins (PV). Alpha-T-catenin co-localized with beta-catenin, plakophillin-2, and connexin-43 at cell junctions in the WT pulmonary vein, similar to heart. No alpha-T-catenin was detected in KO mice. These PV muscle cells were alpha-smooth muscle actin negative with desmin-positive striations, identifying them as bona fide cardiomyocytes. Alpha-T-catenin null PVs show an increase in size of the cardiomyocyte sheath, an effect that is proportionally similar to the established increase in heart size observed in alpha-T-catenin KO mice. **Conclusions:** We detect alpha-T-catenin in the cardiomyocyte sheath of pulmonary veins, and its loss is associated with an enlargement of this muscle layer. How alpha-T-catenin and PV structure contribute to TDI-induced asthma will be determined in a mouse model with the WT and alpha-T-catenin KO mice.

This work is funded by R01HL094643 to CJG.

62

p53 and ATF4 Mediate Distinct and Additive Pathways to Skeletal Muscle Atrophy During Limb Immobilization

***DK Fox*[*], KS Bongers[*], SM Ebert[*], MC Dyle[*], SA Bullard[*,†], JM Dierdorff[*], SD Kunkel[*], CM Adams[*,†]**
[*]University of Iowa, Departments of Molecular Physiology and Biophysics and Internal Medicine and Fraternal Order of Eagles Diabetes Research Center, Iowa City, IA; [†]Iowa City Veterans Affairs Medical Center, Iowa City, IA

Immobilization causes skeletal muscle atrophy via complex molecular mechanisms that are not well understood. To better understand these mechanisms, we investigated and compared the roles of p53 and ATF4, two transcription factors that mediate adaptations to a wide variety of cellular stresses. Previous studies established that muscle immobilization increases skeletal muscle ATF4 expression, which is sufficient to induce muscle fiber atrophy. However, skeletal muscles lacking ATF4 are only partially resistant to muscle atrophy during limb immobilization, indicating the existence of an ATF4-independent pathway. Here, we demonstrate that p53 mediates the ATF4-independent pathway to muscle atrophy during limb immobilization. Using mouse models, we show that limb immobilization increases p53 expression in skeletal muscle, and forced expression of p53 in skeletal muscle fibers is sufficient to induce muscle fiber atrophy. Conversely, mice lacking p53 expression in skeletal muscle fibers are partially resistant to immobilization-induced skeletal muscle atrophy. Importantly, p53 promotes muscle fiber atrophy in the absence of ATF4, whereas ATF4 promotes muscle fiber atrophy in the absence of p53. Furthermore, forced expression of both p53 and ATF4 induces more muscle fiber atrophy than either p53 alone or ATF4 alone. In addition, skeletal muscle lacking both p53 and ATF4 is more resistant to immobilization-induced muscle atrophy than muscle lacking only p53 or ATF4. Collectively, these results demonstrate that p53 and ATF4 mediate distinct and additive pathways to skeletal muscle atrophy during limb immobilization.

63

Caenorhabditis elegans as a *Mycobacterium marinum* Virulence Model

***T Galbadage*[*], SLG Cirillo[*], TL Gumienny[†], JD Cirillo[*]**
Departments of [*]Microbial Pathogenesis and Immunology, and [†]Molecular and Cellular Medicine, Texas A&M University Health Science Center, Bryan, TX

Mycobacterium marinum are pathogenic mycobacteria that cause cutaneous granulomatous lesions in humans and a chronic tuberculosis-like granulomatous disease in ectotherms, including fish and amphibians. *M. marinum* are commonly used to understand *M. tuberculosis (Mtb)* virulence and pathogenesis. They are genetically closely related to *Mtb*, share many virulence genes, cause granulomas and are much easier to handle in the laboratory compared to *Mtb*. However, there are limitations to the use of *M. marinum* as a model for *Mtb* because *M. marinum* have an optimum growth temperature of <33°C. *Caenorhabditis elegans* are used as a host to study various pathogens including *Pseudomonas*, *Staphylococcus*, *Legionella*, *Enterococcus*,

Shigella and *Proteus* species because of their ease of use and genetic tractability. *C. elegans* have a well characterized innate immune system. Extensive mutant and RNAi libraries exist for analysis of their immune responses, making *C. elegans* a very attractive model. We fed *C. elegans* with *M. marinum* and characterized nematode morbidity and mortality. *C. elegans* fed on *M. marinum* for 24 hours display a mortality rate of >80% within two days post-infection. In contrast, nematodes fed on the non-pathogenic mycobacterial species *M. smegmatis* have a mortality rate of <10%. Using fluorescent tagged *C.* elegans, *M. marinum* and *M. smegmatis* we observed that there is also a difference in localization of pathogenic and non-pathogenic mycobacterial strains, suggesting that *C. elegans* are readily colonized by pathogenic mycobacteria. Virulent *M. marinum* persist in the pharyngeal, gut and tail regions of the nematode, while avirulent *M. smegmatis* are easily broken down or cleared from the gut. Furthermore, *C. elegans* display different temporal and morphological characteristics based on virulence of the mycobacterial strain they are exposed to. Our observations demonstrate that *M. marinum* are pathogenic to *C. elegans* and suggest that these nematodes can be used for analysis of *M. marinum* virulence. We evaluated the versatility of *C. elegans* as a virulence model for *M. marinum* by infecting them with an array of *M. marinum* mutants with macrophage infection defects. Several mutants were significantly less virulent in *C. elegans* causing reduced mortality rates of 40-65%, suggesting related mechanisms of infection in macrophages and *C. elegans*. This study establishes *C. elegans* as a new model for analysis of mycobacterial virulence and mechanisms of host immune response.

65

Prospective Interventional Pilot Study Using Bromfenac 0.07% After Cataract Surgery for the Prevention of Macular Thickening

***Ivayla I Geneva*[1], Bonnie A Henderson[2,3]**

[1]State University of New York – Upstate Medical University; [2]Tufts University School of Medicine; [3]Ophthalmic Consultants of Boston

Purpose: To evaluate the effectiveness of topical bromfenac 0.07% for the prevention of macular thickness after cataract surgery. **Methods:** In a prospective interventional study, 38 consecutive eyes of 25 patients, mean age 72.2 years +/- 12.9 [SD], underwent uncomplicated phacoemulsification and received perioperative and post-operative loteprednol etabonate ophthalmic gel 0.5% QID taper and bromfenac 0.07% qday for 4 weeks postoperatively and besifloxacin ophthalmic suspension 0.6% TID for 1 week. Pre-operatively and at 3-4 weeks post-operatively macular OCTs and best-corrected visual acuity (BCVA) were obtained. Cells and flare were assessed one day and one month post-operatively. The OCT-based change in retinal thickness post-surgery was compared with values in the literature from trials where established regimens were used. **Results**: The mean pre-op and post-op thickness of the 38 eyes studied were 258.2 +/- 17.9 [SD] and 259.5 +/- 18.5, respectively. The mean change in macular thickness was 1.26 +/- 2.98 [SD], range [-11 to 5] which is less than when using other published regimens. There was a slight decrease in pre-operative macular thickness with age (r = -0.18) but no correlation between age and the change in thickness after phacoemulsification was observed (r = 0.08). The improvement of BCVA showed slight negative correlation with change in macular thickness (r = -0.19). The post-operative intraocular pressure ranged from 12 to 30mmHg, with decrease to below the pre-operative level by one month. **Conclusions**: Our prospective interventional pilot study demonstrate that bromfenac 0.07% in combination with topical loteprednolol following uncomplicated phacoemulsifications can effectively prevent macular thickening, eliminate anterior chamber inflammation, and result in improved visual acuity to levels comparable to, and sometimes better than, previously studied post-operative regimen. A larger study is needed in order to better understand the potential benefits offered by this lower bromfenac preparation in terms of the potential reduction in the NSAID-related side effects.

66

Advanced Diffusion MRI for the Development of Neuroimaging Biomarkers in Alzheimer's Disease

***GR Glenn,* JA Helpern, JH Jensen**

Medical University of South Carolina, Charleston, SC

Alzheimer's disease (AD) is a major public health concern which is expected to get worse as the population ages. There is currently no known cure or treatment to slow the progression of AD and the only FDA-approved treatments provide symptomatic relief but do not slow the underlying disease process. As a result, research is urgently needed to better understand the underlying pathogenic mechanisms. To facilitate research, there is a pressing need to develop biomarkers to aid in the early detection of AD and to provide sensitive markers of disease severity as outcomes measures in clinical trials of novel therapeutics. Quantitative white matter (WM) neuroimaging techniques such as diffusion tensor imaging (DTI) and diffusional kurtosis imaging (DKI) have demonstrated potential to provide early biomarkers of AD by measuring microstructurally-related neurodegenerative changes that result from a variety of possible molecular mechanisms in AD. Recently, kurtosis-based WM fiber tractography was developed by our lab as an extension of DKI to allow advanced fiber tracking with clinically feasible diffusion MRI image datasets, while simultaneously quantifying biologically meaningful properties of complex WM tissue microstructure. Kurtosis-based tractography enables detection of multiple fiber bundle orientations in a single image volume element to track white matter fiber bundles through complex neural environments, which is not possible with DTI alone. This is expected to provide substantial improvements in the ability to map neural connectivity and visualize specific WM fiber tracts non-invasively in the human brain which may be affected by disease. A method for performing advanced, kurtosis-based fiber tractography will be presented. The method has been developed and optimized in a small cohort of 5 healthy volunteers. With kurtosis-based tractography, we can reproducibly resolve crossing fibers in anatomical regions with known crossing fibers, such as the pons, where the transverse pontine fibers of the middle cerebellar peduncle interdigitate between ascending and descending fiber tracts between the brainstem and higher brain regions at nearly right angles. The methods we have developed can also be applied for the comprehensive characterization of WM tissue microstructure along specific tracts known to be affected in

Poster Abstracts

AD, such as the corpus callosum, the fornix, and the cingulum bundle. This work is currently being researched in a larger cohort of 41 subjects recruited to study pathogenic mechanisms of neurodegeneration and the development of neuroimaging biomarkers in AD.

67

Aurora A Kinase is Required for Hematopoiesis and Couples Polyploidization with Terminal Differentiation in Megakaryocytes Through Phosphorylation of NF-E2

B Goldenson, **QJ Wen, J Crispino**
Medicine, Division of Hematology/Oncology, Northwestern University, Chicago, IL

We have recently shown that small molecule inhibitors of Aurora A kinase (AURKA) induce polyploidization and differentiation of normal and malignant megakaryocytes. To determine the mechanism by which AURKA inhibitors ameliorate the leukemic phenotype, we have examined the functional requirement for *Aurka* in adult hematopoiesis. Complete loss of AURKA in hematopoietic cells caused a rapid and profound defect in hematopoiesis. Notably, we observe an increase in the proportion of CD41 and CD42-positive megakaryocytes. To determine whether the observed defects are cell autonomous, we transplanted *Aurka*$^{fl/fl}$, Mx1-Cre bone marrow cells to lethally irradiated recipients. Upon AURKA deletion, the transplanted mice develop an identical phenotype to the one observed in the conditional knockout mice. Moreover, in competitive transplantation experiments, Aurka$^{-/-}$ cells fail to contribute to hematopoiesis. Mechanistically, loss of AURKA led to a significant degree of apoptosis in hematopoietic cells. We next deleted AURKA in megakaryocytes ex vivo and found that deletion of AURKA resulted in increased CD41 and CD42 expression as well as increased ploidy of the megakaryocyte fraction. To investigate whether AURKA modulates differentiation of megakaryocytes through interactions with lineage specific transcription factors, we performed co-IP experiments between AURKA and megakaryocyte transcription factors. Results confirmed a robust interaction between AURKA and p45 NF-E2. Additionally, we found by an in vitro kinase assay that AURKA phosphorylates p45 NF-E2 on the S170 residue. Overexpression of wild-type NF-E2 significantly increased the megakaryocyte population, while overexpression of the S170D mutant was less effective in promoting megakaryocyte differentiation, indicating that the loss of phosphorylation promotes NF-E2 activity. Finally, we knocked-down down NF-E2 and treated with Aurora A inhibitors. Strikingly, cells with NF-E2 knocked-down displayed significantly less differentiation with AURKA inhibitor treatment compared to control knockdowns. Taken together, our data show that Aurora A kinase is required for adult hematopoiesis and that Aurora A regulates NF-E2 function during megakaryocyte differentiation.

68

Targeting UPEC Adhesive Pili Provides Genetic Insight and Potential Therapeutics

***SE Greene*[1], SJ. Hultren[2]**
[1]MD-PhD Program, Washington University in St. Louis, St. Louis, Mo; [2]Department of Molecular Microbiology, Center for Women's Infectious Disease Research Washington University in St. Louis, St. Louis, MO

Adhesion is important in the establishment of many bacterial infections. The increasing prevalence of antibiotic resistance motivates the discovery of new anti-virulence treatments, and one potential treatment strategy targets adhesive pili. Uropathogenic *E. coli* (UPEC) are the major cause of urinary tract infections (UTI), one of the most common infections in women. UPEC use type 1 pili with the FimH tip adhesin, to bind mannosylated glycoproteins on the luminal bladder surface, facilitating bacterial colonization and invasion of the bladder epithelium. Type 1 pili are assembled by a chaperone and usher, which enable proper pilus subunit folding in the periplasm and polymerization through the outer membrane, respectively. Thus, type 1 pili are termed chaperone usher pathway (CUP) pili. Each sequenced UPEC strain encode a multitude of homologous CUP pilus operons, and the most studied of these, type 1, P and S pili are associated with cystitis, pyelonephritis and neonatal meningitis, respectively. Here, we characterize potential therapeutics, termed pilicides that target these adhesive pili. Pilicides were designed to disrupt the function of the chaperone, preventing proper pilus assembly. We demonstrate that our pilicides are effective at altering CUP subunit expression and pilus assembly. We characterize two classes of pilicides that have different effects on UPEC CUP pili and that work through different mechanisms. One class appears to bind to and alter the pilus chaperone while the other might work through interactions with the global regulator Crp. Both these mechanisms could prove fruitful in treating UPEC UTIs or other infections mediated by CUP pili in different gram-negative bacteria.

69

Rapid Experience-Dependent Changes in Visual Responses

***TC Griffen*[*‡], A Fontanini[†], A Maffei[†‡]**
Program in Neuroscience; [*]Medical Scientist Training Program;[†]Department of Neurobiology and Behavior, Stony Brook University, Stony Brook, NY; [‡]SUNY Eye Institute, NY

Prolonged loss of visual input to one eye leads to an inability to perceive stimuli delivered to the deprived eye. In rodents, closure of one eye by lid suture for several days shifts visual responses of cortical neurons to favor the open eye. Two hypotheses have been proposed to explain this effect: 1) selective weakening of excitatory inputs to cortex or 2) strengthening of inhibition within cortex. To determine the cause of lost visual responsiveness, most investigations have focused on examining the function of the visual cortical circuit after several days of monocular deprivation. However, changes in synaptic strength and spontaneous firing within layer 4, the primary input layer of cortex, can be observed in as little as 24 hours after the onset of the visual manipulation. At this time point, changes in peak visual responsiveness within layer 4 have not been observed. Here, we use in vivo whole cell

recordings to examine baseline neuronal activity in cortex as well as single neuron responses to visual stimuli following 24 hours of unilateral visual deprivation. We find changes in the time course of neuronal responses to brief visual stimuli very rapidly following the onset of the visual manipulation. Our results suggest that a combination of changes in excitatory and inhibitory drive may contribute to alter neuronal function. The results obtained from this work will be instrumental to understanding the mechanisms driving the initial stages of change in cortical response induced by altered visual drive, and may provide new opportunities for designing therapeutic strategies for the recovery of visual function.

70

Regulation of Endothelial NF-κB Activity by Methionine Oxidation

***SX Gu*, JW Stevens, AK Chauhan, SR Lentz**
University of Iowa Carver College of Medicine, Iowa City, IA

Cardiovascular diseases such as atherosclerosis, vasculitis, and thrombosis are associated with increased production of vascular reactive oxygen species (ROS) that can oxidize protein methionine residues. Reversible methionine oxidation allows proteins containing redox-sensitive methionine residues to respond to altered cellular redox balance during normal homeostasis, but dysregulated methionine oxidation can alter protein function and contribute to pathophysiology. The endothelial NF-κB pathway regulates the expression of cytokines, adhesion molecules, and growth factors involved in the pathogeneses of vascular disease. Prior studies suggest that methionine residues in NF-κB regulatory proteins are susceptible to oxidation. We therefore tested the hypotheses that ROS such as H_2O_2 promote the activation of endothelial NF-κB and that methionine sulfoxide reductase (MSR), which reverses protein methionine oxidation, protects against ROS-augmented NF-κB activation. Using an adenoviral NF-κB reporter construct, we found that H_2O_2 potentiated TNFα-induced NF-κB activity by 30% ($P < 0.05$) in human umbilical vein endothelial cells. H_2O_2-augmented NF-κB activation was prevented by overexpression of MSR type A but not MSR types B1 or B3A. To determine if NF-κB activity is regulated by methionine oxidation *in vivo*, MSRA-deficient (*MsrA-/-*) mice were crossbred with HLL transgenic mice that express *Photinus* luciferase under the control of an NF-κB-dependent promoter. We found that MSRA deficiency resulted in tissue specific increases in NF-κB activation in the lung, aorta, and heart of *MsrA-/-* mice ($P < 0.05$). The luciferase reporter protein was found to co-localize with the endothelial marker protein CD31 in carotid arteries by immunofluorescence confocal imaging. P-selectin-dependent leukocyte rolling was significantly increased in the mesenteric venules of *MsrA-/-* mice compared with wild-type mice (122 ± 22 vs. 60 ± 7 leukocytes/min; $P < 0.05$). Finally, susceptibility to stroke was assessed using a middle cerebral artery occlusion (MCAO) model of ischemia-reperfusion injury. We found a 40% increase in cerebral infarct volume in *MsrA-/-* mice compared with wild-type mice ($P < 0.05$). We conclude that endothelial NF-κB activity is regulated by reversible methionine oxidation *in vitro* and *in vivo* and that MSRA-deficient mice have increased vascular inflammation and increased susceptibility to ischemic stroke. These findings suggest that oxidation of methionine residues in NF-κB regulatory proteins may represent a modifiable process that contributes to the proinflammatory and prothrombotic phenotype of cardiovascular disease.

71

Regulation of Cardiac Progenitor Cell Differentiation by Hdac3

***M Gupta*[*], MM Lu[*], CM Trivedi[†], JA Epstein[*]**
[*]Department of Cell and Developmental Biology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA; [†]Department of Medicine, University of Massachusetts Medical School, Worcester, MA

Recent studies have shown that class 1 histone deacetylases (HDACs) are critical regulators of cardiac morphology and function, sparking an interest to develop therapeutics targeting class 1 HDACs for the treatment of heart disease. However, little is known about the function of certain members of this important class of epigenetic modifiers during the early stages of cardiac development. In particular, Hdac3 has been shown to regulate the cardiac response to high fat diet in adult hearts, however little is known about its role in embryonic cardiac development and its function in cardiac progenitor cells. We hypothesized that Hdac3 regulates differentiation and expansion of cardiac progenitors during development. Using a floxed *Hdac3* allele and several cardiac–specific *Cre* mouse lines, we genetically deleted *Hdac3* in *Nkx2.5*-, *Isl1*-, *SM22*α-, and α*MHC*-expressing cells. The Hdac3-null mice exhibited ventricular hypoplasia and embryonic lethality when we deleted *Hdac3* in multipotent cardiac progenitor cells, before cardiomyocytes are specified. Using flow cytometry, we determined that cardiomyocytes made up a higher percentage of the cells in *Hdac3*-null hearts relative to wild-type controls, suggesting that loss of *Hdac3* promotes a preferential differentiation of cardiac progenitor cells into cardiomyocytes. Additionally, we observed a similar preference for differentiation towards cardiomyocytes at the expense of other cardiac lineages in mouse ES cells in which we deleted *Hdac3* during embryoid body-mediated cardiac differentiation. Together, these data suggest a model in which Hdac3 regulates the differentiation of multipotent cardiac progenitor cells during early development by preventing early, preferential differentiation towards the cardiomyocyte lineage.

72

H3K36 Methyltransferase Loss Results in Widespread RNA Processing Defects and Increased Tumor-Promoting Phenotypes in Human Renal Cell Carcinoma

***KE Hacker*[*], JM Simon[*], CC Fahey[*], D Singh[*], AR Brannon[*], JS Parker[*], M Weiser[*], TH Ho[†], PF Kuan[*], E Jonasch[†], TS Furey[*], JF Prins[*], JD Lieb[*], IJ Davis[*], and WK Rathmell[*]**
[*]University of North Carolina at Chapel Hill, Chapel Hill, NC; [†]MD Anderson, Houston, TX

Comprehensive sequencing of human cancers has identified frequent mutations in chromatin regulatory proteins, highlighting the importance of chromatin maintenance in tumor suppression. Specifically, the only human histone H3K36 trimethyltransferase, SETD2, is mutated in a wide variety of tumor types, including

clear cell renal cell carcinoma (ccRCC). To understand how SETD2 mutations promote cancer growth or development, we undertook an innovative and comprehensive genomic analysis by performing Formaldehyde-Assisted Isolation of Regulatory Elements (FAIRE-seq) across a large cohort of primary ccRCC tumors and matched uninvolved kidney. FAIRE-seq interrogates chromatin structure by enriching for nucleosome-depleted DNA, which is associated with gene regulatory activity in normal cells. Using this approach, we linked *SETD2* mutations with nucleosome loss at genes normally marked by H3K36 tri-methylation, which is globally eliminated in *SETD2* mutant tumors. We then investigated the effects of H3K36me3 deficiency on the ccRCC transcriptome and observed RNA processing defects, including intron retention and alternative splicing, within pre-mRNA transcripts that persisted into the mature mRNA pool. Remarkably, these defects affected approximately 25% of all expressed genes and occurred at transcripts marked by H3K36me3 in normal kidney and by nucleosome depletion in H3K36me3-deficient tumors. These striking results suggest that RNA processing defects are associated with H3K36me3 loss and severely deregulate a wide variety of important cellular pathways. To further investigate the consequences of SETD2 mutations on tumor growth, we used TAL-Effector Nucleases (TALENs) to inactivate SETD2 in human renal cell lines and subsequently re-introduced disease-relevant SETD2 mutants. As expected, SETD2 inactivation resulted in global H3K36me3 loss and recapitulated RNA processing defects observed in human tumors. Additionally, SETD2 loss significantly enhanced tumor-promoting properties, including increased cell proliferation, anchorage-independent growth, and migration. This mechanistic link between defects in chromatin organization, aberrant RNA processing, and tumor-promoting phenotypes provides crucial evidence for how recurrent mutations in chromatin regulatory proteins promote cancer and identifies novel targets with therapeutic potential to improve patient care.

73

The Spatial Organization of Chemosensory Information Modulates Functional Responses in the Mouse Accessory Olfactory Bulb

***GF Hammen*[1,2], JP Meeks[3], and TE Holy[1]**

[1]Department of Anatomy & Neurobiology, Washington University in St. Louis, St. Louis, MO; [2]MD-PhD Program, Washington University in St. Louis, St. Louis, MO; [3]The University of Texas Southwestern Medical Center, Dallas, TX

We used the mammalian accessory olfactory system (AOS) is as a model system for sensory processing. The peripheral neurons of the mouse AOS are the vomeronasal sensory neurons (VSNs). The VSN cell bodies lie within the vomeronasal organ along the septal floor of the nasopharynx and project axons to glomerular structures in the accessory olfactory. The size of the system makes it tractable to record from the entire circuit and make inferences about neural processing. Our initial investigations of the AOB utilized two techniques record stimulus responses across the AOB. First we utilized a genetically encoded calcium indicator, GCaMP2, in the VSN population. Second, we rapidly imaged the VSN axon terminals in the AOB glomerular layer using objective-coupled planar illumination (OCPI) microscopy. Together these allowed us to characterize the location and functional characteristics of much of the AOB input layer. We delivered sulfated steroid stimuli to the VSNs within the vomeronasal organ and recorded fluorescence changes at the AOB glomeruli. We used these fluorescence changes to create a map for sulfated steroid stimuli within the AOB. To determine whether presynaptic feedback influenced response map at this early stage, we investigated the effects of glutamatergic and GABAergic neurotransmission on glomerular functional responses. Coactivation of adjacent glomeruli by mixed stimuli resulted in suppression of responses in these glomeruli, a form of lateral inhibition. Antagonism of postsynaptic ionotropic glutamate receptors abrogated this circuit mediated lateral inhibition. Antagonism of presynaptic GABA(B) receptors reduced inhibitory feedback resulting in larger presynaptic responses and blocked lateral inhibition.. These results indicate that GABA(B) feedback at these first synapses occurs during normal neurotransmission and shapes the functional response map during presentation of complex mixtures.

74

Activation of Toll-Like Receptor 2 Enhances Contractile Response from Adrenergic Alpha-2 Receptor Stimulation in Mesenteric Vessels

Trevor Hardigan*, Maria Alicia Carrillo-Sepulveda, R. Clinton Webb

**Georgia Regents University, Augusta, GA*

Toll-like receptors (TLRs) are pattern recognition receptors of the innate immune system that recognize microbial pathogens as well as endogenous damage-associated molecular patterns (DAMPS). TLR2 has been linked to a variety of cardiovascular diseases such as atherosclerosis and heart failure, but its role in vascular function is unknown. Preliminary data from our lab suggests that stimulation of TLR2 with the synthetic agonist Pam3CSK4 increases sensitivity to adrenergic stimulation with norepinephrine. We hypothesized that this response was due to an effect on alpha-2 receptors as opposed to alpha-1 receptors. We used second order mesenteric arteries from 15-week old Sprague Dawley rats in a small vessel wire myograph. Concentration response curves to alpha-1 agonist phenylephrine (Phe; 10^{-9}-10^{-4} M) and alpha-2 agonist clonidine (Clon; 10^{-9}-10^{-4} M) were performed in vessels incubated with and without TLR2 agonist Pam3CSK4 [(P3C4); 500ng/mL; 30› prior incubation]. TLR2 stimulation in the treatment vessels did not have an effect on the alpha 1 response vs. control (EMax as % of maximum KCl response: 128.4±5.92% vs. 120.5±5.25%, respectively). In contrast, contraction with clonidine in the presence of P3CSK4 was significantly increased vs. control (EMax as % of maximum KCl response: 68.7±11.6% vs. 35.3±12.2%, respectively). These results indicate that acute activation of TLR2 has increases the contractile response to alpha-2 stimulation, potentially contributing to vascular dysfunction in resistance arteries.

Poster Abstracts

75

Redox Modulation of STAT6 and Jmjd3 in the Development of Pulmonary Fibrosis

***Chao He*[2], Alan J Ryan[1], Shubha Murthy[1], A Brent Carter[1,2,3,4]**

Departments of [1]Internal Medicine; [2]Radiation Oncology and Graduate Program in Free Radical and Radiation Biology, Carver College of Medicine; [3]Graduate Program in Human Toxicology, College of Public Health, University of Iowa; and [4]Iowa City Veterans Administration Healthcare System, Iowa City, IA

M2-phenotype macrophages contribute to fibrotic development. We have shown that independent of Th2 cytokines, Cu,Zn-SOD-mediated H_2O_2 generation promotes M2 polarization by regulating STAT6. In the current study, we hypothesize that STAT6 up-regulates M2 gene expression by augmentation of expression and activity of the demethylase, Jmjd3. Jmjd3 has been linked to activation of M2 genes by demethylation of histone 3-lysine 27. Macrophages isolated from Cu,Zn-SOD^{Tg} mice have significantly increased *jmjd3* gene expression and Jmjd3 enzymatic activity compared to macrophages from the WT mice. We have previously showed that STAT6 activation requires a redox regulatory Cys^{528} residue in the SH2 domain. The expression of *jmjd3* is abolished in cells expressing Cys^{528} mutant compared to cells expressing an empty vector. Moreover, Jmjd3 enzymatic activity is upregulated in alveolar macrophages from patients with pulmonary fibrosis compared with normal subjects. Because Jmjd3-mediated M2 gene activation is downstream of STAT6, we tested our hypothesis in STAT6$^{-/-}$ mice. As expected, STAT6$^{-/-}$ mice have a significant decrease in *jmjd3* expression that results in a dramatic decrease in M2 markers expression in alveolar macrophages. Furthermore, STAT6$^{-/-}$ mice are protected from developing pulmonary fibrosis compared with WT mice. To explore a potential modulator of STAT6 and to translate the importance of STAT6 *in vivo*, we utilized leflunomide, an inhibitor of STAT6. We found that alveolar macrophages isolated from leflunomide-treated mice have significantly decreased *jmjd3* and M2 gene expression compared with vehicle-treated mice. Importantly, mice treated with leflunomide have attenuation of fibrosis compared with mice treated with vehicle. These observations suggest that STAT6-mediated epigenetic regulation of M2 genes is important for pulmonary fibrosis development, and uncover leflunomide as a potential therapeutic agent to prevent progression of pulmonary fibrosis.

76

Mechanism of Action of rpL13a snoRNAs in Lipotoxicity

***MH Henderson,* SJ Langmade, JE Schaffer**

Washington University School of Medicine, St. Louis, MO

Lipid overload in non-adipose tissues is associated with organ dysfunction in obese and diabetic humans and in animal models of these diseases. On a cellular level, exposure to pathophysiological levels of lipid induces oxidative and endoplasmic reticulum stress. A genetic screen using promoter trap mutagenesis identified a role for the *rpL13a* locus in lipotoxic cell death. Complementation and knockdown studies demonstrated that three intronic small nucleolar RNAs (snoRNAs), U32a, U33, and U35a, but not the rpL13a protein, are the critical mediators of lipotoxicity within the *rpL13a* locus. While canonical snoRNAs localize to the nucleolus and direct chemical modification of ribosomal RNAs (rRNAs) and small nuclear RNAs, the *rpL13a* snoRNAs accumulate in the cytoplasm during lipotoxic stress and their putative rRNA targets are not differentially modulated, suggesting they may play a noncanonical role in lipotoxicity. This study focuses on characterization of the mechanism of action of the *rpL13a* snoRNAs through characterization of the ribonucleoproteins (RNPs) they form. We used biotinylated antisense oligo-nucleotides to isolate complexes containing U33 from whole cell sonicates. In addition to the canonical snoRNP proteins fibrillarin, Nop56, and Nop58, U33 specifically associates with additional proteins that may play a role in the non-canonical localization and/or function of this snoRNA and may have relevance for the complications of obesity and diabetes.

77

Proteopathic Tau Seeding is an Early and Robust Predictor of Tauopathy

***Brandon B. Holmes*[†‡], Jenny L. Furman[†], Tritia R. Yamasaki[†], Nigel J. Cairns[†], Marc I. Diamond[†]**

[†]Department of Neurology, Washington University in St. Louis; [‡]MD-PhD Program, Washington University in St. Louis

Tauopathies are a class of neurodegenerative disorders characterized by the pathological accumulation of microtubule-associated protein tau in the human brain. In the early stage of disease, tau pathology is often restricted to discrete and stereotyped regions of the brain. With disease progression, however, the pathological changes typically spread through the nervous system according to specific anatomical patterns. Emerging evidence demonstrates that tau aggregates are capable of transcellular spread whereby an aggregate formed in one cell is released freely into the extracellular space, enters a neighboring cell, and converts the natively folded tau protein into an aggregated, fibrillar form. This phenomenon, termed *seeding*, predicts that misfolded tau aggregates capable of transcellular spread directly serve as an agent of disease progression. Thus, a highly sensitive and selective methodology for the quantitative detection of tau seeds in cells, animal models, and patient materials would be welcomed. Here, we describe a novel and facile seeding assay that reliably detects tau seeds from mouse and human brain homogenates. We engineered a FRET biosensor cell line expressing human P301S mutant tau. Proteopathic tau seeds transduced into this biosensor cell line induce intracellular aggregation which is measured via FRET flow cytometry. Using this cell-based seeding assay, we can quantitatively detect recombinant tau seeds in the femtomolar range. We employed this approach to demonstrate that seeding activity in brain homogenates of the P301S mouse model is an early and robust predictor of tauopathy. For example, the P301S mouse model displays tau histopathology beginning at 4 months of age as characterized by CP13, AT8, and MC1 antibody staining; canonical neurofibrillary tangles commence at approximately 9 months of age. However, we here report that tau seeding activity in these mice begins at 1.5 months of age and progressively increases over time. These findings establish tau seeding as the earliest pathological change to date. Finally, we employed this assay to detect seeds in the brains of human patients. Alzheimer disease,

corticobasal degeneration, and Pick's disease brain homogenates display robust seeding activity in our assay, while Parkinson's and Huntington's disease brains do not. In sum, we have established a highly quantitative and facile seeding assay that holds promise as a future diagnostic assay for tauopathies.

78

Oncogenic Myc Disrupts NAMPT Circadian Oscillation in Mouse Hepatocellular Carcinoma Cell Line

***AL Hsieh*[*], BJ Altman[†], A Venkataraman[†], DI Bellovin[‡], DW Felsher[‡], JB Hogenesch[†], and CV Dang[†]**

[*]JHMI, Baltimore, MD; [†]UPenn, Philadelphia, PA; [‡]Stanford University, Stanford, CA

MYC, which is overexpressed in a variety of human cancers, drives aerobic glycolysis, also called the Warburg effect. The Warburg effect results in regeneration of NAD^+ from increased conversion of pyruvate to lactate, such that decreased lactate dehydrogenase A (LDHA) activity results in NAD^+ depletion and can result in cell death. Nicotinamide phosphoribosyltransferase (NAMPT), a rate-limiting enzyme that is involved in the NAD^+ salvage pathway, was reported to be directly upregulated by Myc. Increased NAD^+ synthesis thus helps to replenish NAD^+ along with LDHA. In this regard, we have previously reported the synergy of inhibiting LDHA and NAMPT in causing lymphoma xenograft regression. Targeting NAMPT by its inhibitor FK866 has been tested in clinical trials. However, the toxicity of FK866 in normal cells is not negligible, with thrombocytopenia being reported. Toxicity, however, could be diminished if therapy is administered at specific times of the day (termed chronotherapy) when normal cells are least dependent on NAMPT, whose levels oscillate every 24 hours. We have previously found that Myc upregulates Rev-erbα, which inhibits and disrupts oscillation of the circadian rhythm gene ARNTL (protein name Bmal1) in the osteosarcoma cell line U2OS. However, although NAMPT was shown oscillating in normal mouse liver and other tissues, how the oscillation of NAMPT is affected in cancer especially under Myc overexpression is still poorly understood. Here we show Myc induces Nampt mRNA and protein in mouse hepatocellular carcinoma (mHCC) cell line that has been engineered tetracycline inducible Myc transgene expression vector. Inhibition of NAMPT by FK866 induces perturbation of circadian gene expression in mHCC cells, including Rev-erbα, Per1 and Cry1. The alteration of circadian gene expression can be rescued by nicotinamide, suggesting that the circadian clock is affected by NAD^+/NADH ratio. Interestingly, Nampt mRNA oscillates in mHCC cell lines in the absence of Myc and the oscillation was lost when Myc is induced, suggesting a therapeutic opportunity could exist in the window between circadian regulation of the ebb-and-flow of normal cell NAMPT level and the sustained, non-circadian cancer cell NAMPT level. Our observation that Myc disrupts the oscillation its target gene NAMPT provides a conceptual framework for metabolic chronotherapy that could potentially lead to better cancer treatment strategies that reduce side effects.

We thank the following founding sources: NIH R01CA57341, LLS 6106-14.

79

Cerebral Performance Category at Hospital Discharge Predicts Long-Term Survival of Cardiac Arrest Survivors Receiving Targeted Temperature Management

***Cindy H. Hsu*, Jiaqi Li, Marisa J. Cinousis, Kelsey R. Sheak, David F. Gaieski, Benjamin S. Abella, Marion Leary**

Department of Emergency Medicine and Center for Resuscitation Science, Perelman School of Medicine, University of Pennsylvania

Objective: Despite recent advancements in post-cardiac arrest resuscitation, the optimal measurement of post-arrest outcome remains unclear. We hypothesized that cerebral performance category (CPC) score can predict the long-term outcome of post-arrest survivors who received targeted temperature management (TTM) during their post-arrest hospital care. **Design:** We performed a retrospective chart review of cardiac arrest patients at two academic medical centers from May, 2005 to December, 2012. The CPC scores at hospital discharge were determined by three independent abstractors. The 1-month, 6-month, and 12-month survival of these patients were determined by reviewing hospital records, querying the Social Security Death Index, and follow-up telephone calls. Kaplan-Meier survival estimates were calculated to compare unadjusted rates of long-term survival. Cox proportion hazards regression model was used to estimate survival associated with CPC after adjusting for relevant covariates. **Main Results:** Of the 2,417 identified cardiac arrest patients, 24.1% (582/2417) were successfully resuscitated, of whom 24.1% (140/582) received post-arrest TTM. Overall, 42.9% (60/140) were discharged with CPC 1, 27.1% (38/140) with CPC 2, 18.6% (26/140) with CPC 3, and 11.4% (16/140) with CPC 4. CPC 1 survivors had the highest long-term survival followed by CPC 2 and 3, with CPC 4 having the lowest long-term survival ($p < 0.001$, log-rank test). We found that CPC 4 remained associated with worse survival (hazard ratio = 12.73, $p < 0.001$) while CPC 3 demonstrated a trend toward worse survival (hazard ratio = 3.41, $p = 0.068$) after adjusting for age, gender, race, shockable rhythm, time to TTM, total duration of resuscitation, withdrawal of care, and out of hospital arrest. **Conclusion:** Patients with different CPC scores at discharge have significantly different survival trajectories. Favorable CPC at hospital discharge predicts better long-term outcomes of cardiac arrest survivors who received TTM than those with less favorable CPC scores.

80

Characterization of G-Protein Coupled Estrogen Receptor Expression in Zebrafish Embryos

***Muhan Hu*[1,3] and Daniel Gorelick[2,3]**

[1]Medical Scientist Training Program; [2]Department of Pharmacology and Toxicology; [3]University of Alabama at Birmingham, Birmingham, AL

Estrogens act on diverse tissues by binding to multiple estrogen receptors. Studies have focused on the characterization of the classical estrogen receptors (ERa, ERb), which are ligand-dependent transcription factors. Less is known about the more recently discovered G-protein coupled estrogen receptor, which is a membrane associated estrogen receptor. In adult rodents, GPER may influence heart rate and protect the heart following

ischemia. However, less is known about GPER expression and function during embryonic development. Two previous studies reported widespread *gper* expression in zebrafish embryos, including in the heart. However, specific localization of *gper* in the heart was not reported. In this study, we examined the expression of *gper* more closely to identify the specific areas of the heart that express GPER (e.g. heart valves, myocardial cells). This will help us generate a hypothesis about its role in the heart during embryonic development. We performed whole mount *in situ* hybridization on wild type zebrafish embryos at 25 – 96 hours post fertilization. Surprisingly, we did not detect *gper* expression in the embryonic heart. Unlike the widespread *gper* expression reported previously, our results showed restricted *gper* expression in discrete regions in the brain. *gper* transcripts were observed in the presumptive hypothalamus. Bilaterally symmetric *gper* mRNA labeling was also observed in the olfactory bulb and preoptic area in the forebrain. Our results suggest that GPER expression in embryos is not as extensive as previously thought, and that GPER does not act on the heart directly during embryonic development. We hypothesize that GPER activation mediates embryonic heart function via the GPER expressing cells in the brain. Future studies will identify the specific cell types that express *gper* by performing double-label *in situ* hybridization using *gper* and cell-specific probes. We will also identify GPER function by generating *gper* mutant zebrafish.

81

Heme Oxygenase-1 Expression Prevents Doxorubicin-Induced Delayed Onset Heart Failure

***TD Hull*†, Y Wu*, SH Litovsky‡, SD Prabhu*, A Agarwal*, JF George*†**

*University of Alabama at Birmingham, Birmingham, AL. Departments of *Medicine, †Surgery, and ‡Pathology*

Background: Heme oxygenase-1 (HO-1) is an inducible enzyme that degrades pro-oxidant heme into carbon monoxide, biliverdin, and iron. We showed that cardiac-specific HO-1 expression prevents heart failure secondary to acute cardiac injury induced by Cre recombinase. However, the role of HO-1 in delayed onset heart failure (DOHF) caused by the chemotherapeutic agent doxorubicin (DOX) is unknown. **Hypothesis**: Expression of HO-1 protects against DOX-induced DOHF by preventing cardiomyocyte death secondary to mitochondrial toxicity. **Methods and Results**: We used DOX to develop a model of DOHF in mice (18 mg DOX per kg of body weight, administered IV as three 6 mg/kg doses, each separated by two days). This treatment protocol does not cause acute cardiac dysfunction, but results in DOHF as determined by echocardiography at days 14 and 60 after treatment. HO-1 overexpression in humanized transgenic (HBAC) mice prevents body weight loss (4% vs 15% in WT, $P < 0.05$, n=10) and systolic dysfunction (ejection fraction 67% vs 51% in WT mice, $P<0.05$, n=5) at day 14 after DOX treatment. DOX-induced DOHF, characterized by left ventricle dilation (3.22 mm vs 3.55 mm in WT mice, $P<0.05$, n=5) and wall thinning (0.89 mm vs 0.61 mm in WT mice, $P<0.05$, n=5), is also prevented by HO-1 overexpression. Histological evaluation demonstrated that global and cardiac-specific overexpression of HO-1 prevents cardiomyocyte necrosis, evidenced by a reduction in cytoplasmic vacuolization and loss of myocyte striations, as well as secondary inflammation in necrotic foci. DOX increases the proportion of $CD45^+$ cells in the heart of WT mice relative to vehicle treated controls (1.42% vs 0.62%, $P < 0.01$, n=5), while HO-1 expression inhibits the infiltration of $CD11b^+$ mononuclear phagocytes ($P < 0.05$, n=5). HO-1 overexpression also decreases myocardial fibrosis detected by picrosirus staining 60 days after DOX treatment, which is exacerbated in HO-1 deficient mice. Transmission electron microscopy demonstrated that cardiac-specific overexpression of HO-1 ameliorates DOX-mediated ultrastructural changes to cardiomyocytes by preventing dilatation of the sarcoplasmic reticulum, cytoplasmic vacuolization, and myofibrillar disarray at days 14 and 60 after treatment. **Conclusion**: HO-1 expression prevents DOX-induced delayed onset cardiac failure.

82

High Sensitivity Molecular MRI Using a Two-Dimensional Non-Covalent Delivery Vehicle

***AH Hung*, RJ Holbrook, C Glasscock, MW Rotz, KW MacRenaris, N Mansukhani, MC Duch, KT Dam, LM Manus, MC Hersam, and TJ Meade**

Northwestern University, Evanston, IL

Molecular imaging is the non-invasive visualization of biomolecular and cellular processes *in vivo*. Utilization of this technology has grown over the past decade with the increasing demand for molecular information in clinical decision-making. MRI is an attractive molecular imaging modality due to its unmatched spatiotemporal resolution and lack of ionizing radiation. However, contrast agents used in MRI to report on the molecular and cellular activities of interest suffer from an inherent limitation in sensitivity. Nanomaterials with contrast agent payloads have recently emerged to address the issue of sensitivity, but this approach has been met with its own challenges. Nano-conjugates are more complicated to synthesize and characterize compared to their molecular counterparts, and more fundamentally, the colloidal nature of nanomaterials limits their solution concentration. *In vitro*, low concentration constrains the degree of contrast agent labeling in cells and their subsequent monitoring by imaging; *in vivo*, the limitation often preclude the dosages necessary to cross the sensitivity threshold. Graphene oxide (GO) is a two-dimensional carbon nanomaterial functionalized with hydroxyls, carboxylic acids, and epoxides. Its surface-to-volume ratio is the highest of any known material, and its surface chemistry supports every major type of non-covalent interaction. We hypothesize that GO can act as a non-covalent carrier of small molecules simply via co-incubation *in vitro* and co-injection *in vivo* to increase the delivery of molecules to the target cell or tissue. We found that when gadolinium contrast agents were incubated with cells in culture in the presence of GO, the cellular gadolinium payload increased by up to 13 fold compared to when the contrast agent was used alone. This effect is dose-dependent and correlates with the degree of non-covalent interaction between the contrast agent and GO. The strategy circumvented the synthetic complexities of nano-conjugates and allowed for contrast agent concentrations well into the millimolar range during incubation. The resulting enhancement in cell labeling directly increased imaging sensitivity and is expected to expand the utility of cell tracking by MRI.

Poster Abstracts

84

PDE5 Inhibitors Reduce Colitis by Suppressing Apoptosis in the Colon Mucosa

Bianca Islam, **Rui Wang, and Darren Browning**
Department of Biochemistry and Molecular Biology, Georgia Regents University, Medical College of Georgia, Augusta, GA

The cyclic guanosine monophosphate (cGMP) signaling axis controls homeostasis in the intestinal mucosa and has been implicated in the suppression of colitis and colon cancer. There is therefore considerable interest in exploiting this pathway but the signaling downstream of cGMP is poorly defined and a viable therapeutic strategy remains to be established. The objective of the present study was to determine whether the phosphodiesterase-5 (PDE-5) inhibitor Vardenafil (Levitra™), could activate cGMP signaling in the colon mucosa and determine the effect on an experimental model of colitis in mice. Vardenafil was administered to mice by intraperitoneal injection and the colons removed for analysis of cGMP content and protein kinase G (PKG) activation. The effect of Vardenafil on apoptosis in the colon mucosa of wild type and PKG-deficient mice was measured by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL). Activation of cJun N-terminal Kinase (JNK) was measured using immunofluorescence. The therapeutic effect of Vardenafil on colitis in mice was assessed using the dextran sulfate-sodium (DSS) model. Systemic administration of Vardenafil rapidly increased cGMP and activated PKG in the colon epithelium. Vardenafil treatment over several days reduced JNK activity and apoptosis in the luminal epithelium. Consistent with the importance of PKG2 in this process, Vardenafil treatment did not affect apoptosis in PKG2-deficient animals. This augmentation of the epithelial resilience by Vardenafil was protective against DSS-induced damage to the colon mucosa as measured using both gross disease and histological indices. In summary, results shown here demonstrate that treatment with the PDE-5 inhibitor Vardenafil activates cGMP signaling in the colon epithelium. Importantly, our data demonstrates the therapeutic potential of these agents as part of a novel strategy for treating colitis and colon cancer prevention.

85

Bottom-Up Network Pharmacology: Novel Integration of Drug-Target Predictions for Drug Repurposing

NT Issa*, **J Kruger, OJ Peters, SW Byers, and S Dakshanamurthy**
**Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington, DC*

Drug development efforts have been plagued with escalating costs and diminishing outputs. Drug repurposing offers a potential solution-approved drugs are applied to new and possibly unrelated clinical indications. Since approved drugs have known pharmacological profiles, repurposing allows for streamlining into phase II trials. We previously developed a computational proteochemometric drug-target (DT) prediction method entitled "Train, Match, Fit, Streamline" (TMFS) to precisely predict DT binding signatures and formulate repurposing hypotheses. Here we expand the utility of TMFS for integration into networks pharmacology, thus expanding molecular-level DT predictions to the levels of protein function, signaling pathways and clinical disease. As TMFS employs a "bottom-up" approach, previously unknown DT predictions are made and drive higher-order network associations. This model correctly predicted more than 50 drug-protein-disease associations across multiple clinical categories such as cardiovascular, neurologic and oncologic illnesses. Application of networks-based TMFS allows for both discovery of novel targeted therapeutics as well as the prediction of off-target effects. With respect to the former, TMFS predicted mebendazole, an antiparastitic, to have anticancer properties through the binding and inhibition of VEGFR2 kinase activity. We experimentally determined that mebendazole was able to bind VEGFR2 and inhibit angiogenesis. TMFS also predicted the anti-inflammatory compound dimethyl celecoxib to bind cadherin-11, an adhesion molecule implicated in a variety of autoimmune diseases, such as rheumatoid arthritis, as well as poor prognosis malignancies. Surface plasmon resonance confirmed the association between dimethyl celecoxib and cadherin-11. Furthermore, we predicted the binding of the antiviral oseltamivir to the adenosine A2A receptor, possibly establishing the link between this drug and its behavioral side effects. TMFS is a drug discovery platform that places empirical DT predictions within the context of network pharmacology. While current drug repurposing efforts utilize "top-down" approaches from known drug-disease interactions, TMFS is novel in its "bottom-up" methodology, where DT predictions drive drug-disease inferences. Given the completely *in silico* nature of this process, TMFS is poised to increase drug discovery efficiency and ultimately enhance repurposing efforts for clinical success.

86

Hopx$^+$, Type I Cells Act as a Reserve Pool to Regenerate Type II Cells in the Lung in Times of Injury

Rajan Jain**[1,2,3]****, Christina E. Barkauskas[4], Norifumi Takeda[1,2,3], Qiaohong Wang[1,2,3], Haig Aghajanian[1,2,3], Lauren J. Manderfield[1,2,3], Mudit Gupta[1,2,3], Arun Padmanabhan[1,2,3], Deqiang Li[1,2,3], Li Li[2], Brigid L. Hogan[5], Jonathan A. Epstein[1,2,3]**
[1]Department of Cell and Developmental Biology; [2]Penn Cardiovascular Institute; [3]Institute of Regenerative Medicine, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA; [4]Division of Pulmonary, Allergy, and Critical Care Medicine, Department of Medicine; [5]Department of Cell Biology, Duke University Medical Center, Durham, NC

Relationships between tissue-specific adult stem cells and their differentiated progeny during homeostatic conditions and times of regeneration are an area of intense research. Alveolar Type II cells, in addition to secreting surfactant, can function as a progenitor population in the adult lung, while Type I cells function to facilitate gas exchange. However, the ability of Type I cells to give rise to alternate lineages during regeneration has not been reported. We demonstrate that *Hopx*-expression during embryonic lung development marks a bipotent alveolar cell capable of giving rise to both Type I and II cells. In the adult alveoli, *Hopx* expression is restricted to Type I cells. However, we show that during lung regeneration following injury adult *Hopx*$^+$ cells give rise to both Type I and Type II cells. Single adult *Hopx*$^+$, Type I cells can give

rise to alveospheres composed of Type II cells *ex-vivo*. These findings suggest that a bi-directional lineage relationship exists between Type I and II cells and that adult Type I alveolar cells possess unanticipated plasticity.

87

Targeting Polo-Like Kinase 1 (PLK1) Can Overcome Estrogen-Independent Growth in ER+ Human Breast Cancer

N Bhola, *VM Jansen,* S Bafna, J Balko, T Dugger, CL Arteaga
Department of Medicine, Vanderbilt-Ingram Comprehensive Cancer Center; Vanderbilt University, Nashville, TN

Estrogen receptor α (ER)-positive metastatic breast cancers initially respond to antiestrogens, but eventually become estrogen-independent and recur. ER+ breast cancer cells resistant to long-term estrogen deprivation (LTED) exhibit hormone/estrogen-independent ER transcriptional activity and growth. We sought to identify kinases essential for both ligand-independent ER transcriptional activity and growth of ER+ LTED breast cancer cells. A kinome-wide siRNA screen using a library targeting 791 kinases identified polo like kinase 1 (PLK1) siRNA as one of the top hits for inhibition of ligand-independent ER transcriptional activity and growth of MCF7/LTED cells. RNAi knockdown of PLK1 inhibited growth of 2 MCF7 and HCC1428 cell lines and prevented the emergence of hormone-independent cells. PLK1 knockdown inhibited estrogen-independent ER transcriptional reporter activity in MCF7/LTED cells and this effect was specific to ER. Quantitative real-time PCR showed PLK1 knockdown-mediated downregulation of mRNA expression of the ER-regulated genes PR, TFF1 and GREB1. Pharmacological inhibition of PLK1 with BI6727 (volasertib) decreased LTED cell growth, ER transcriptional activity, and ER expression. BI6727 combined with the ER downregulator fulvestrant significantly decreased growth and ER expression in MCF7 xenografts compared to either BI6727 or fulvestrant alone. Results from a PCR array of 84 ER-associated genes showed that BI6727 treatment decreased expression of JUNB, FOS, and BCL2L in MCF7/LTED cells. Interestingly, these 3 genes were upregulated in MCF7 cells treated with BI6727. These data support a critical role of PLK1 for acquired hormone-independent ER transcriptional activity and thereby growth of hormone-independent ER+ human breast cancer cells.

88

Muscle Specific TFAM Overexpression Decreases Bodyweight and Increases Mitochondrial Lipid Oxidation while Reducing Hydrogen Peroxide Production at Baseline and in Response to High Fat Feeding

***ML Johnson,* GC Henderson, S Cooper, D Jakaitis, IR Lanza, KS Nair**
Mayo Clinic, Rochester, MN

We determined if a muscle specific overexpression of mitochondrial transcription factor-A (TFAM) alters body composition and mitochondrial function compared to wild type (WT) controls and whether they respond differently to a high fat diet. WT and TFAM overexpression mice were fed ad libitum a standard (Chow) diet for 13 weeks. Bodyweight and food consumption were measured throughout the time period. TFAM mice (26±0.02g) were significantly smaller than WT mice (30±0.04g). TFAM mice (24±0.02kcal/hr/kg) exhibited elevated whole body metabolic rate in comparison to WT mice (18±0.03kcal/hr/kg) without showing any difference in total activity level. High resolution respirometry of isolated mitochondria revealed that TFAM mice had depressed state 3, complex 1-respiration, and elevated state 3 complex 2-respiration in comparison to WT controls ($P<0.05$) with glutamate/malate substrates. TFAM mice exhibited increased proton leak but reduced H202 production rates compared to WT mice ($P<0.05$) with glutamate/malate substrates. However, analysis of isolated mitochondria with palmitoyl-carnitine substrates revealed significantly elevated state 3 respiration and decreased proton leak ($P<0.05$). High fat feeding (60% of calories from fat, HFD) significantly increased total bodyweight in both WT and TFAM (37±0.05g and 31±0.03g respectively), however TFAM mice gained less body fat ($P<0.05$). HFD significantly increased state 3 respirations of both glutamate/malate substrates and palmitoyl-carnitine substrates in WT and TFAM mice. HFD significantly increased H202 emissions in WT, but not TFAM mice ($P<0.05$) while proton leak remained unchanged. In conclusion, skeletal muscle TFAM overexpression enhanced mitochondrial respiratory efficiency to oxidize lipids and reduced whole body adipose accumulation following a HFD.

89

Long-Lasting Immune Tolerance Mediated by Dendritic Cells Depends on Functions of Homeodomain Only Protein in Peripherally Induced Regulatory T Cells

***Andrew Jones*[1], Adeleye Opejin[1], Cindy Gross[1], Richard A. Flavell[2,3], and Daniel Hawiger[1*]**
[1]Department of Molecular Microbiology and Immunology, Saint Louis University School of Medicine, St. Louis, MO; [2]Department of Immunobiology; and [3]Howard Hughes Medical Institute; Yale University School of Medicine, New Haven, CT

The task of silencing the autoimmune responses mediated by autoreactive T cells is a complex process referred to as immune tolerance that begins in the thymus, continues in the peripheral lymphoid system and relies on T cell selection mechanisms as well as the functions of thymically and peripherally developed regulatory T cells. Peripheral T cell tolerance involves antigen-mediated interactions between T cells and antigen presenting cells including Dendritic Cells (DCs). T cell tolerance induced by DCs can avert autoimmune responses including experimental acute encephalomyelitis (EAE), a model of multiple sclerosis (MS). This DC-induced tolerance includes multiple mechanisms to inhibit such autoimmune responses by blocking immune activation of antigen-specific T cells. However, the specific mechanisms by which DCs induce long-lasting tolerance preventing future autoimmune injury remain unknown. We recently discovered Homeodomain Only Protein (Hopx) to direct Treg cell-mediated immune unresponsiveness induced by DCs. Here we show that DC-mediated long-lasting tolerance depends on Hopx-regulated inhibition of IL-2 expression in peripheral regulatory T cells (pTreg cells) that are induced extrathymically. Loss of Hopx abrogates long-lasting tolerance induced within 3 weeks after targeting myelin oligodendrocyte glycoprotein (MOG) to DCs under steady

Poster Abstracts

state conditions. During that time pTreg cells develop and these pTreg cells are then required and sufficient to block autoimmune EAE following immunization with MOG. Therefore our results further clarify a division of labor between nTreg cells and pTreg cells by showing a unique requirement for pTreg cells in long-lasting tolerance induced by DCs to neural antigens. Moreover, we identify Hopx-dependent tolerogenic mechanism in such pTreg cells by revealing that absence of Hopx results in IL-2 production by these cells and such self-made IL-2 disrupts maintenance of pTreg cells and tolerance.

90

A 51-Year-Old African American Man Presenting with Syncope and Fever

Venkat R. Kakollu, **Larisa Mararenko, Mohammad H. Zaman**
Brookdale University Hospital and Medical Center, Brooklyn, NY

Introduction: First reported in 1989, ICL was formally described by the CDC in 1992. CDC criteria to diagnose ICL include, "CD4+ T-lymphocyte depletion, no serologic evidence of HIV infection, and absence of any defined immunodeficiency or therapy associated with T-cell depletion." ICL is a rare disorder of unknown etiology and posed a diagnostic challenge in our case. **Case Presentation:** A 51-year-old African American man presented to the emergency department (ED) with an episode of syncope and fever. Patient was hemodynamically stable. On physical exam, patient was confused, disoriented and had bilateral lower extremity weakness (3/5), normal reflexes, unsteady gait, urinary incontinence, scattered maculo-papular rash over extremities and abdominal wall. **Diagnostic Testing:** Admission labs revealed Pancytopenia, Reactive RPR and elevated D-dimer level. CXR showed non-specific bilateral hilar opacities. CT head demonstrated a moderate communicating hydrocephalus. Lumbar puncture revealed elevated protein of 322mg/dl, WBC of 6, glucose of <20 mg/dl, xanthochromia and positive cryptococcal antigen. CT Chest and abdomen showed mediastinal and hilar adenopathy, numerous 'air' cysts in upper lobes, pulmonary nodules, marked splenomegaly, upper abdominal and retroperitoneal adenopathy. Due to the presence of reactive RPR and positive cryptococcal antigen in the CSF, was negative. Lymphocyte Panel revealed profoundly low absolute CD4/CD8 counts of (77/28cells/$mm^{3)}$ with a normal CD4/CD8 Ratio. Also, patient had elevated ACE level and a non-reactive P-24 antigen. Skin Biopsy revealed findings consistent with Sarcoidosis. Lung Biopsy showed findings consistent with Pulmonary Cryptococcosis. Celiac Lymph Node Biopsy and Bone marrow biopsy showed Non-Necrotizing Granulomas and Acid-Fast Bacilli (AFB). Patient was treated with Amphotericin-B/Flucytosine/PenicillinG/INH/Rifampin/Ethambutol/Pyrizinamide and Clarithromycin, with significant clinical improvement and was discharged. **Conclusion:** Initially we attributed low CD4/CD8 counts to Sarcoidosis. However, the presence of two opportunistic infections: AFB in bone marrow and celiac lymph node and disseminated cryptococcus led to the diagnosis of ICL. The presence of opportunistic infections in a HIV-negative patient should lead to an investigation for the possibility of ICL. Currently, the association between Sarcoidosis and ICL is unknown and needs further research.

91

Genetic Mechanisms of Chandelier Interneuron Specification in Mouse Cerebral Cortex

S.M. Kelly*, **M. He****, **Z. J. Huang****
[*]Stony Brook University, Stony Brook, NY; Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Neuronal firing activity within the cerebral cortex is regulated by a diverse assortment of inhibitory GABAergic interneurons, which are able to finely tune circuit activity through a vast array of morphological, molecular, synaptic, and electrophysiological properties. Most cortical interneurons in rodents arise predominantly from three neurogenic zones: the medial and caudal ganglionic eminences (MGE, CGE), and the preoptic area (POA). There is evidence that interneuron class identity is determined in part in part by spatial and temporal origin within the subpallium. Furthermore, intrinsic genetic programs and extrinsic cortical signals are both likely to play a role in defining different neuronal subtypes and distributing these cells to cortical layers and regions in the proper density to allow the formation of meaningful circuit motifs. To approach a complete understanding of interneuron development, our studies have focused on characterizing the entire life history of the chandelier cell, arguably the most distinct and uniform GABAergic interneuron yet to be described. Chandelier cells have an unmistakable axon arbor of hundreds of vertically arranged cartridge synapses, each of which specifically innervates the axon initial segment of a pyramidal cell, the site where action potentials are generated. Neocortical chandelier cells (ChCs) originate from Nkx2.1+ progenitors in the ventral germinal zone (VGZ), a previously uncharacterized progenitor region below the lateral ventricle. Here, using intersectional genetic fate mapping and novel birth dating techniques, we provide an experimental system to examine the cellular and molecular nature of progenitors that produce a well-defined cell type in cerebral cortex. Further, we have compared the birth timing and progenitor origin of ChCs in various regions of the cerebrum, such as the hippocampus, piriform cortex, and neocortex. We hope to discover and characterize distinct subpopulations of Nkx2.1+ progenitors that distribute ChCs to major cerebral subdivisions and thus give rise to functional ChC subtypes via temporal regulation in the MGE and VGZ during mid-to-late gestation. ChC number and synapse development have been shown to be deficient in schizophrenia patients. Therefore, by studying these events, we hope to begin to understand how disordered neuronal circuit assembly during embryonic brain development ultimately manifests as a constellation of debilitating cognitive and behavioral disturbances in schizophrenia, an illness that afflicts as much as 1% of the general population.

92

The Role of β3-Integrin in Breast Cancer Metastases to the Brain

C. Kersch*, **S. Lal, L.L. Muldoon, E.A. Neuwelt**
[*]Oregon Health and Science University, Portland, OR

Background: HER2-positive breast cancers often metastasize to the brain, limiting treatment options. Invasion of the brain with metastases requires neoplastic cells to navigate through the

blood-brain barrier and brain microenvironment. Integrins are a family of transmembrane adhesion receptors that facilitate cell-cell and cell-ECM interactions, and the αvβ3 integrin dimer is common in brain metastases. We hypothesize β3-integrin is important in binding of breast cancer cells to brain vasculature and/or infiltration into brain parenchyma. This project aimed to study the role of β3-integrin in cell proliferation, migration, invasion, and interaction with the growth factor receptor HER2 in a brain-seeking breast cancer cell line. **Methods and Results:** β3-integrin was stably knocked down by shRNA transduction in a human breast cancer cell line MDA-MB231BR that was previously transfected to overexpress HER2. Clonal cell lines with high or low levels of β3-integrin in combination with high or low levels of HER2 were isolated, and protein expression levels were confirmed by western blot. Serial cell viability assays showed that knockdown of β3-integrin or HER2 deficiency alone had minor effects on cell growth, but cells lacking both β3-integrin and HER2 showed an increased proliferation rate. Reduced expression of β3-integrin alone decreased migration 80% and invasion 77%, as assessed by transwell infiltration without or with Matrigel. Reduced expression of both β3-integrin and HER2 decreased migration and invasion 91% and 88%, respectively. Immunoprecipitation experiments showed that β3-integrin and HER2 co-precipitated, suggesting they complex in cells. To assess *in vivo* effects of β3-integrin on tumor morphology, the β3-integrin knockdown cell clones were intracranially inoculated in athymic rats. Brains were harvested 4 weeks post inoculation for analysis of tumor growth and infiltration into the brain parenchyma. **Conclusions and Future Directions:** Initial results suggest that the β3 subunit of integrin interacts with HER2 in brain-seeking breast cancer cells, which negatively affects their capacity for migration and invasion. Further studies are in process to better elucidate this interaction, including immunofluorescence studies to confirm co-localization of β3-integrin and HER2 in the cell, and intracarotid injection of these knockdown cell lines in athymic rats to assess changes in their hematogenous spread. We suggest that β3-integrin may provide a therapeutic target in breast cancer brain metastases.

93

Novel Molecular Engineering Approaches to Reveal Mechanisms of Proper Brain Cell Development in Adult Brain Tissue

***CJ Kim*[*,§], I Garcia[*,§], BA Arenkiel[*,§]**

[*]Baylor College of Medicine, Houston, TX; [§]Jan and Dan Duncan Neurological Research Institute at Texas Children's Hospital, Houston, TX

Background: Growth of the aged population has been accompanied by increased prevalence of neurodegenerative diseases. Pharmaceutical therapies for conditions such as Parkinson's and Alzheimer's Diseases have contributed significantly to symptomatic management, but at their best, these compounds slow the effects of neurodegeneration rather than halting disease progression. More potent approaches to treatment include methods of cell type-specific replacement or repair, which hold promise of reversing degeneration and restoring function to debilitated circuitry. Despite burgeoning investigation into stem cell transplants and gene therapy, however, much remains unknown about the requirements of *de novo* synaptogenesis in pre-established adult brain tissue. Remarkably, new synapses are formed daily by adult-born neurons in the olfactory circuit of the mammalian brain. We exploit this phenomenon by transplanting neural stem cells into the olfactory germinal niche and examining the requirements of proper neuronal development and tissue integration. We previously showed that transplanted neurons derived from mouse embryonic stem cells integrated into the olfactory bulb and established functional synaptic connectivity in intact animal brains. Developing this technology to enable engrafted cells to express genes critical to the maturation of neurons *in vivo* would be of great clinical interest. Here we show that stem cell-derived neurons can be engineered to harbor genetic constructs to allow ectopic expression of any gene of interest. The development of "induced" stem cell technology has further expanded options for sources of brain cell grafts to include the host's own non-neural tissues. We show here further that induced neural progenitor cells can be derived from mouse fibroblasts and, like embryonic stem cell-derived neurons, can be engineered to harbor exogenous elements. These studies illustrate the molecular functionality and modularity of our engineering constructs from within the genomes of various neural cell lines. We ultimately aim to apply this technology towards the development of novel molecular tools, to further understand the genetic basis of neuronal development in the post-natal brain, and to make inroads into new methods of cell replacement and repair in the treatment of a variety of neurodegenerative diseases.

94

Brown Adipose Tissue is Present in Human Newborns with Congenital Hypothyroidism Secondary to Athyreosis

***Mimi S. Kim*[*†], Houchun Hu[*†], Mitchell E. Geffner[*†], Vicente Gilsanz[*†]**

[*]Children's Hospital Los Angeles, University of Southern California, Los Angeles, CA; [†]The Saban Research Institute, Los Angeles, CA

Background: The transition to a colder extra-uterine environment at birth is regulated by the hypothalamus, sympathetic nervous system, and brown adipose tissue (BAT), and involves complex interactions with thyroid hormone. Thyroid hormone receptors, along with type 2 deiodinase (D2) that converts thyroxine (T4) to triiodothyronine (T3), are present in brown adipocytes, and their deletion or inactivation leads to impaired BAT thermogenesis in animals. Newborns born with severe hypothyroidism due to athyreosis represent a unique human population in which to examine BAT in the absence of thyroid hormone. **Methods:** Newborn infants with congenital hypothyroidism were recruited from the Pediatric Endocrinology clinic following identification of an abnormally elevated thyroid stimulating hormone (TSH) level by the California newborn screening program. Prior to levo-thyroxine replacement, each infant underwent a physical exam, blood analytes [free T4, TSH, thyroglobulin], and a magnetic resonance (MR) imaging scan to assess thyroid dysgenesis and provide BAT measures. Chemical-shift water-fat MR technique was used to obtain quantitative measurements of fat and water content (fat fraction) in the supraclavicular adipose depot. Four newborns with thyroid agenesis had a baseline visit and one follow-up assessment at one to three months of age. Data are

presented as mean ± SD, and were analyzed by t-tests for paired samples and linear regression. **Results:** All four athyreotic infants (5.7 ± 0.5 days old) were born to healthy mothers and were products of full-term pregnancies, with initial visit weights of 3.2 ± 0.6 kg, length 48.1 ± 0.7 cm, elevated TSH 445-1220 mIU/L, low fT4 0.27-0.9 ng/dL, and undetectable thyroglobulin (<0.1 ng/mL). At the follow-up visit, weight was 4.8 ± 0.4 kg, length 54.0 ± 1.5 cm, TSH 5.3 ± 8.2 mIU/L, and fT4 3.3 ± 2.2 ng/dL. Although all MRI studies depicted BAT in the supraclavicular area at both visits, follow-up exams showed a decrease in BAT (as indicated by an increase in fat fraction) in the supraclavicular fossa (18.4% ± 7.1 vs. 44.5% ± 14.7; P = 0.007). When all exams were analyzed together, there was a strong positive correlation between TSH levels and the FF of the supraclavicular area (r = 0.78; P = 0.02). In contrast, the FF in the subcutaneous white adipose depot did not significantly change (67.6% ± 12.3 vs. 82.7% ± 4.5; p = 0.058) and was not related to TSH levels (r = 0.38; P = 0.34). **Conclusion:** Large amounts of BAT are present in human newborns with thyroid agenesis, which decrease with T4 replacement. The results of the current study also suggest an association between TSH and BAT. Further work is needed to determine whether TSH stimulates thermogenesis via TSH receptors present on brown adipocytes.

95

The Role of the Crohn's Disease Susceptibility Allele, *ATG16L1^{T300A}*, in Mouse Secretory Lineage Function

***Kleckner VL**[*], **Patel KK**[*], **Liu TC**[*], **Virgin HW**[*], **Xavier RJ**[+], **Stappenbeck TS**[*]*

[*]Washington University School of Medicine St. Louis, MO; [+]Massachusetts General Hospital, Boston, MA

Crohn's Disease (CD) is an inflammatory condition of the intestine that results from a combination of genetic susceptibility and environmental stimuli. The most recent meta-analysis of genome-wide association studies (GWAS) identified 163 susceptibility alleles for inflammatory bowel disease. One of these alleles is relatively specific for CD and encodes a single nucleotide alteration in the ATG16l1 gene that alters that amino acid produced at that location (the T300A mutation). We previously found that mouse models with a complete or partial loss of Atg16l1 function in the intestinal epithelium created profound defects in the susceptibility to pathogen infection as well as the secretion by Paneth cells (antimicrobial proteins) and goblet cells (mucus). We also found that human CD patients with the T300A allele also showed abnormalities in Paneth cells. We generated mice that contained the T300A polymorphism in the mouse Atg16l1 locus. Our goal was to test the effects of the T300A mutation on intestinal secretory lineages in this line of mice and determine if there were environmental factors that could trigger any observed defects. Mice homozygous for the T300A knock-in were compared to age-matched, wild-type littermate control mice using both histological sections of the intestines and cultured cell lines developed from these mice. Histological analysis of colons showed that T300A knock-in mice had enlarged goblet cells at the colonic surface that were similar to the phenotype observed in mice with a complete knockout of Ag16l1 in the intestinal epithelium. We found that this phenotype occurred when mice were infected with murine norovirus indicating a role for this virus as a microbial trigger. No difference in goblet cell size was observed in MNV negative mice or in the cultured colon and small intestinal epithelial cell lines. Our data indicate that the T300A allele is associated with alterations in intestinal secretory cells that can be triggered by viral infection. Further study elucidating the mechanism for these differences will help uncover the pathogenesis of this key mutation in CD.

96

Human Metapneumovirus F Protein Histidine 434 Linked to a Hyperfusogenic Phenotype

***Edita Klimyte*, Andres Chang, and Rebecca E. Dutch**

Department of Biochemistry, University of Kentucky, Lexington, KY

Human metapneumovirus (HMPV) is a recently discovered respiratory pathogen in the *Paramyxoviridae* family that infects nearly 100% of the world population. This enveloped RNA virus causes severe respiratory disease in infants, the elderly, and immunocompromised patients worldwide. In fact, HMPV is estimated to be the second most common cause of pediatric lower respiratory illness, following the closely related respiratory syncytial virus. Furthermore, the rate of hospitalization for respiratory disease caused by HMPV in adults over the age of 65 is near that of influenza. Despite its clinical significance, there is no antiviral treatment or vaccine available at this time. Entry of paramyxoviruses into host cells typically requires the coordinated activity of the attachment glycoprotein G, which interacts with a cell receptor, and the fusion glycoprotein F, which promotes subsequent fusion of viral and cellular membranes. Interestingly, unlike other paramyxoviruses, recombinant HMPV without G is replication competent in cell culture and in multiple animal models, suggesting unique function of HMPV F. The F protein undergoes a dramatic conformational change in order to bring the viral and target cell membranes together. Our group previously showed that HMPV F is triggered to fuse membranes by a low pH pulse for the strain CAN97-83 (Clade A2). It is not well understood which residues in the fusion protein contribute to sensitivity to low pH. We isolated the F protein from a previously uncharacterized HMPV strain by reverse transcriptase polymerase chain reaction. This protein exhibited a hyperfusogenic phenotype, confirmed by independent fusion assays, syncytia formation and luciferase reporter gene assay. Sequence analysis revealed a single residue change, at position 434 to a histidine, between the F protein of CAN97-83 and the hyperfusogenic variant, suggesting this residue causes an increase in sensitivity to low pH. Interestingly, this position is adjacent to a conserved histidine at 435 that has been suggested to play a role in potential electrostatic interactions to induce conformational change. These results indicate this area of the F protein is critical for fusion, supporting previous findings regarding the importance of adjacent residue histidine 435.

Poster Abstracts

97

Adipocyte-Specific Ablation of PPAR-gamma Co-Repressor NCoR Modulates Metabolism and Attenuates Experimental Skin Fibrosis

Benjamin Korman, **Roberta Marangoni, Warren Tourtelotte, John Varga**

Feinberg School of Medicine, Northwestern University, Chicago, IL

Systemic sclerosis (SSc) is characterized by fibrosis in the skin and multiple organs, and is associated with aberrant PPAR-gamma signaling. Skin fibrosis is commonly accompanied by loss of intradermal adipose tissue in SSc, as well as in mouse models of inducible scleroderma. We hypothesized that decreased adipose PPAR-gamma contributes to dermal fibrosis in SSc. To determine the effect of augmented local PPAR-gamma signaling on cutaneous fibrosis, we induced bleomycin dermal fibrosis via adipocyte-specific NCoR ablation. NCoR null mice fed a high fat diet demonstrated activation of PPAR-gamma. These mice showed enhanced insulin sensitivity compared to control mice, displayed alterations in levels of serum adipokines, and were resistant to bleomycin-induced changes in adipocyte size and function and loss of intradermal adipose tissue. Importantly the NCoR knockout mice displayed attenuated skin fibrosis. To assess the role of inflammation on skin fibrosis, we utilized high fat diets with either lard or fish-oil bases and while fish oil was less inflammatory and protective of fibrosis in control animals, this effect was attenuated in NCoR knockout mice. These findings suggest that enhancing adipogenesis via PPAR-gamma can modulate fibrosis in vivo and that adipose tissue therefore plays an active role in the pathogenesis of skin fibrosis. Studies examining metabolism and therapies targeting adipose tissue may therefore represent an important way of targeting fibrosis in SSc.

98

Regulatory T Cells Occupy an Isolated Niche in the Intestine That is Antigen-Independent

***Lisa L. Korn*[1]**, **Harper G. Hubbeling[1], Paige M. Porrett[2], Qi Yang[3], Lisa G. Barnett[1], and Terri M. Laufer[1,4]**

[1]Department of Medicine; [2]Department of Surgery; [3]Department of Pathology and Laboratory Medicine; Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA; [4]Philadelphia Veterans Affairs Medical Center, Philadelphia, PA

CD4+ regulatory T cells (Tregs) maintain immune homeostasis and prevent autoimmunity. T cell receptor-major histocompatibility complex class II signals are necessary for thymic nTreg development and peripheral iTreg generation. However, the requirement for MHCII in Treg homeostasis in tissues such as intestinal lamina propria (LP) is unknown. We examined LP Treg homeostasis in mice in which nTregs develop but lack peripheral TCR-MHCII interactions and no iTregs are generated. nTregs enter the LP and proliferate independently of MHCII in weanlings to fill the compartment. However, new thymic Tregs could access the adult LP and parabiosis showed that Tregs were LP-resident, suggesting a closed niche. This isolated niche was independent of IL-2 but dependent on commensal bacteria. These data demonstrate an LP Treg niche can be filled, isolated, and maintained independently of antigen signals and iTregs. This niche may represent a tissue-specific mechanism to maintain immune tolerance.

99

The CD8α+ Dendritic Cell Specific Gene, Rab43, is Important for Antigen Cross-Presentation and Resistance to *Listeria monocytogenes*

***NM Kretzer*[*†] and KM Murphy[†]**

[*]MD-PhD Program, [†]Dept. of Pathology and Immunology, Washington University, St. Louis, MO

Dendritic cells (DCs) are professional antigen presenting cells that play a critical role in orchestrating the appropriate immune response against foreign pathogens and tumors. Work involving the Batf3 knock-out mouse has substantiated the importance of the CD8α+ DC subset in antigen cross-presentation, which is required for clearance of intracellular pathogens as well as anti-tumor immunity. The lack of CD8α+ DCs in these mice is also associated with resistance to intravenous *Listeria monocytogenes* infection. This resistance led to the "Trojan horse" hypothesis, where *L. monocytogenes* utilizes CD8α+ DCs as an obligate entry site in order to seed infection in the lymphoid follicles of the spleen as well as in the liver. This phenomenon is potentially related to the complex vesicular machinery within the CD8α+ DC, which on one side allows for efficient cross-presentation of antigens and on the other side allows for the survival and the transport of *L. monocytogenes* to the T cell areas of the spleen. In order to identify and characterize unique components of the CD8α+ DC vesicular machinery, we performed microarray analysis on different DC subsets. One of the identified genes, Rab43, is highly and specifically expressed within CD8α+ DCs. Moreover, Rab43 shows over 90% conservation between mouse and human. In order to address the role of Rab43 within CD8α+ DCs, we generated constitutive and conditional knock-out mice. Mice with constitutive deletion of Rab43 are viable and display unaltered hematopoiesis. Although CD8α+ DCs are preserved in numbers, knock-out mice are resistant to *L. monocytogenes* infection, as Batf3 deficient mice. Moreover, the Rab43 knock-out mice have a 100 fold decrease in bacterial burden in the liver and a 10 fold reduction in the spleen. Cytoplasmic entry of bacteria is also decreased in Rab43 knock-out mice, suggesting a defect in a vesicular trafficking pathway. This defect in vesicular trafficking is also evident by a decrease in antigen cross-presentation in the Rab43 KO mice. We are currently generating anti-Rab43 antibodies in order to understand the vesicular pathways responsible for these results. Our findings will significantly improve the understanding of the vesicular specialization within CD8α+ DCs. This knowledge will potentially result in the development of more specific DC-based tumor immunotherapies and improve our understanding of *L. monocytogenes* pathogenesis.

Poster Abstracts

100

Serum Amyloid A Modulates Monocyte Recruitment and Facilitates Early Lesion Development in LDL Receptor Deficient Mice

***P.A. Krishack*[†], C.V. Bhanvadia[‡], J. Lukens[‡], G.S. Getz[†,‡], C.A. Reardon[†,‡]**

[†]*Committee on Molecular Pathogenesis and Molecular Medicine,* [‡]*Department of Pathology, University of Chicago, Chicago, IL*

Atherosclerosis is one of the major underlying causes of myocardial infarctions and strokes. Studies have associated atherosclerosis with increased plasma levels of acute phase proteins, with one of the most prevalent being serum amyloid A (SAA). While several studies have demonstrated a clear correlation between SAA and atherogenesis, it is unknown if SAA is an active participant or simply a biomarker. SAA is produced predominantly by hepatocytes and macrophages. Plasma levels of SAA are significantly upregulated in LDL Receptor Deficient ($LDLR^{-/-}$) mice fed a high fat/high cholesterol Western type diet (WTD). To study the role of SAA in atherosclerosis, our laboratory obtained mice globally deficient in acute phase isoforms SAA1 and SAA2 from Frederick de Beer at the University of Kentucky. $SAA^{-/-}$ mice were crossed to $LDLR^{-/-}$ mice ($SAA^{-/-}LDLR^{-/-}$) and fed a WTD for 6 or 12 weeks. Results indicate that SAA induces a site-specific phenotype, with $SAA^{-/-}LDLR^{-/-}$ mice demonstrating 31% less lesional area in the ascending aorta ($p<0.024$), but not in the aortic root or innominate artery after 6 weeks of WTD. At 6 weeks the lesions are predominantly macrophage foam cells. There was no difference in plasma lipids and lipoprotein profiles. The phenotype is lost in more mature lesions, when both strains are fed WTD for 12 weeks, suggesting that SAA is involved in the development of early lesion formation. In the absence of SAA, there is a significant increase in total and monocyte Ly6c subsets in the blood. THP-1 monocyte chemotaxis was also reduced *in vitro* when plasma from $SAA^{-/-}LDLR^{-/-}$ mice fed WTD was used. To determine if SAA produced by macrophages, induced atherosclerosis bone marrow transfer experiments was performed. $SAA^{-/-}LDLR^{-/-}$ recipients that received $LDLR^{-/-}$ bone marrow had slightly larger lesions than $LDLR^{-/-}$ mice that had received $SAA^{-/-}LDLR^{-/-}$ bone marrow, suggesting that SAA produced by macrophages, induces lesion formation. Collectively these data suggest that SAA may recruit monocytes to lesions and have led us to postulate that SAA is pro-atherogenic affecting early atherogenesis.

101

Enhancing ER-Chaperone Function Attenuates Bleomycin-Induced Lung Fibrosis

***Jonathan A. Kropski*[*], Dong-Sheng Cheng[*], Peter M. Gulleman[*], Linda A. Gleaves[*], Harikrishna Tanjore[*], Lisa R. Young[*], William E. Lawson[*†], Timothy S. Blackwell[*†]**

[*]*Vanderbilt University School of Medicine, Nashville, TN;* [†]*Department of Veterans Affairs Medical Center, Nashville, TN*

Background: Endoplasmic reticulum (ER) stress is common in alveolar epithelial cells (AECs) in Familial Idiopathic Pneumonia (FIP) caused by surfactant protein C (*SFTPC*) mutations and sporadic idiopathic pulmonary fibrosis (IPF). Humans and mice harboring mutations in *SFTPC* and Hermansky-Pudlak Syndrome 2 (HPS2) have increased risk of lung fibrosis, but ER stress is thought to contribute prominently only in *SFTPC* mutations. We hypothesized that 4-phenylbutyrate (4-PBA), which reduces ER stress in a number of models, would enhance ER chaperone function and reduce bleomycin-induced lung fibrosis specifically in a model of increased AEC ER stress. **Methods:** Type II AEC-specific, tetracycline-inducible L188Q *SFTPC* expression or wild-type (WT) control mice (C57BL/6) were administered doxycycline for 7 days to induce transgene expression then administered 0.04 units bleomycin (BLM) by intratracheal (IT) injection. 4-PBA (2g/L) in drinking water or placebo was begun day 7 after BLM. 4-PBA or placebo were started one day before HPS2 or control mice were administered IT BLM. Lung fibrosis was analyzed 7-21 days after BLM by modified fibrosis score and hydroxyproline content. ER stress was analyzed by XBP-1 splicing by RT-PCR and Bip mRNA expression by qPCR. **Results:** In L188Q *SFTPC* mice, 4-PBA treatment decreased XBP1 splicing and Bip mRNA expression compared to placebo. 4-PBA treated L188Q *SFTPC* mice also had significantly reduced fibrosis scores and hydroxyproline content and a trend toward improved lung compliance compared to placebo-treated L188Q *SFTPC* mice. Nonsignificant trends in decreased markers of ER stress and lung fibrosis were seen in PBA-treated WT mice. In contrast, there was no difference in survival, weight loss, histologic fibrosis or collagen content in 4-PBA treated HPS2 mice. **Conclusions:** Treatment with the ER chaperone 4-PBA decreases markers of ER stress in the lung and reduces fibrotic remodeling following bleomycin exposure in mice expressing mutant *SFTPC*. ER stress modulation represents a rational therapeutic strategy for further study in patients with FIP caused by surfactant protein mutations and could be broadly effective in all forms of IPF.

Poster Abstracts

102

Investigation of Pathological Response to Neoadjuvant Chemotherapy in Breast Cancer Patients Using Near-Infrared Diffuse Optical Spectroscopic Imaging

***S. Kukreti*[*†‡], A. Cerussi[†], A. Leproux[†], Y. Santoro[†], D. Hsiang[†], R. Mehta[†], B. Tromberg[†], and E. Gratton[‡]**

[*]Department of Radiology, University of California Los Angeles Medical Center, Los Angeles, CA; [†]Beckman Laser Institute and Medical Clinic, UC-Irvine Medical Center, University of California-Irvine, Irvine CA; [‡]Laboratory for Fluorescence Dynamics, Department of Biomedical Engineering, University of California-Irvine, Irvine, CA

In patients with locally-advanced breast cancer, neoadjuvant chemotherapy can produce a complete pathological response, which is an important endpoint that correlates with survival. Because many patients do not respond to neoadjuvant chemotherapy, and conventional clinical assessments do not adequately predict pathological response, there is interest in developing imaging methods to monitor and predict response both prior to and during treatment. Diffuse Optical Spectroscopic Imaging (DOSI) provides a non-radiation view of tissue metabolic state based on the intrinsic near-infrared absorption contrast of hemoglobin, bulk lipid and water. Tissues undergoing neoadjuvant chemotherapy display changes in these molecules, but these molecules are not cancer specific. A self-referencing differential spectroscopy analysis of DOSI revealed spectral signatures thought to represent dispositional shifts in NIR absorbers that may be more specific for malignancy. Can these spectral signatures evaluate pathological response in breast cancer tissue treated with neoadjuvant chemotherapy? DOSI methods were utilized to quantitatively measure absorption spectra in 2D grids centered upon the known tumor location. Research was performed at the Beckman Laser Institute and Medical Clinic at UC-Irvine. The subjects were women, pre- and post-menopausal, diagnosed with biopsy-confirmed breast cancer and scheduled for neoadjuvant chemotherapy. DOSI-measured absorption spectra were used to calculate hemoglobin, water, and bulk lipids concentrations/states as well as the tumor spectral signatures. Tumor spectral signatures were present in all breast tumors and revealed inter-tumor spatial variation, suggesting heterogeneity. The specific tumor component index and heterogeneity index quantified this variation by characterizing the spectral features. Following treatment, changes in these spectral features correlated with pathological response.DOSI-measured spectroscopic methods provide tissue functional characterization that may predict pathological response to neoadjuvant chemotherapy. Information derived from tumor spectral/spatial heterogeneity may improve the detection of residual disease and improve pathological response predictions. DOSI may complement conventional anatomic imaging methods to provide an accessible technology capable of evaluating tumor response to breast cancer treatments such as neoadjuvant chemotherapy.

103

A High Throughput Screen for Modulators of Neuronal Manganese Status

***Kevin K. Kumar*[*], Asad A. Aboud[*], Mihir V. Odak[*], Michael Uhouse[*], John X. Sun[*], M. Diana Neely[*], Michael Aschner[†], Aaron B. Bowman[*]**

[1]Dept of Neurology, Vanderbilt University, Nashville TN; [2]Dept of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY

There is strong evidence that both genetic and environmental factors play a critical role in the pathogenesis of many neurodegenerative diseases. Exposure to the heavy metal, manganese (Mn), shows a strong correlation with incidence of Parkinson's disease (PD). This study presents the results of a high-throughput screen (HTS) to identify novel small molecule modifiers (SMMs) of neuronal Mn accumulation. We screened multiple chemical libraries including: SPECTRUM, NIH Clinical Collection, ChemBridge/Div, and kinase inhibitors from Roche, GSK, and Enzo. Our goal is to identify agents that define mechanisms of Mn regulation and that have clinical potential in modifying Mn environmental risk. We have optimized the Cellular Fura-2 Manganese Extraction Assay (CFMEA) for HTS (Z-factor>0.5) in an immortalized murine striatal cell line ($STHdh^{Q7/Q7}$). Subsequently, we conducted a screen of over 40,000 SMMs in the context of a pathophysiological (125μM) Mn exposure. Hits from this screen were confirmed and subsequently validated through generation of concentration response curves (CRCs). CRCs revealed concentration-responsiveness for 129 of SMM hits. Medicinal chemistry analysis identified 28 of CRC-validated SMMs passed the Lipinski criteria, indicating high *in vivo* translational potential for these compounds. In addition, validated SMMs were tested in the context of a physiological Mn exposure (31.25μM), revealing 77 molecules with the ability to modulate basal Mn levels. Known targets of identified SMMs include cellular signaling pathways previously implicated in Mn toxicity, GSK3β, ALK, EGFR/ErbB2, ERK, and neurotransmitter trafficking proteins. Our current efforts focus on the characterization of SMMs for activity in human induced pluripotent stem cell (hiPSC)-derived mesencephalic dopaminergic neural progenitors. In addition, we are performing *in vivo* studies in *C. elegans* to assess the ability of identified SMMs to modulate tissue Mn levels as measured by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS).

Support: NIH/NIEHS RO1 ES010563 (MA and ABB) and RO1 ES016931 (ABB).

104

Protein Tyrosine Phosphatase Shp2 and Neonatal Cardiac Innervation

***JD Lajiness*[1], P Snider[1], SJ Conway[1]**

[1]Indiana University School of Medicine, Indianapolis, IN

Autonomic innervation is an essential component of cardiovascular regulation that is first established from the neural crest (NC) lineage *in utero* and continues developing postnatally. While *in vitro* studies have indicated Src homology protein tyrosine phosphatase 2 (Shp2) is a signaling factor critical for regulating sympathetic neuron differentiation, this has yet to be shown in the complex *in vivo* environment of cardiac autonomic innervation. Targeting Shp2 within post-migratory NC lineages resulted in a

Poster Abstracts

fully penetrant mouse model of diminished sympathetic cardiac innervation and concomitant bradycardia. Immunohistochemistry of the sympathetic nerve marker tyrosine hydroxylase revealed a progressive loss of adrenergic ganglionic neurons and reduction of cardiac sympathetic axon density in *Shp2* cKOs. Molecularly, *Shp2* cKOs exhibit lineage-specific suppression of activated phospo-ERK1/2 signaling, but not of other downstream targets of Shp2 such as AKT. Genetic restoration of the pERK deficiency via lineage-specific expression of constitutively active MEK1 was sufficient to rescue the sympathetic innervation deficit and its physiological consequences. These data indicate that Shp2 signaling specifically through pERK in post-migratory NC lineages is essential for development and maintenance of sympathetic cardiac innervation postnatally.

105

Rev-Erb Represses Gene Regulation by Inhibiting Enhancer-Directed Transcript

***MT Lam*[†], H Cho[‡], HP Lesch[†], D Gosselin[†], S Heinz[†], Y Tanaka-Oishi[†], C Benner[†], MU Kaikkonen[†], AS Kim[*], M Kosaka[†], CY Lee[†], A Watt[*], TR Grossman[*], MG Rosenfeld[†], RM Evans[‡], CK Glass[†]**

*[†]UC San Diego, La Jolla, CA; [‡] Salk Institute for Biological Studies, La Jolla, CA; *Isis Pharmaceutical Inc, Carlsbad, CA*

Enhancers are DNA regulatory elements bound by lineage-determining and signal-specific transcription factors that facilitates gene regulation in a spatial and temporal fashion. Recently, active enhancers have been shown to be transcribing RNA pervasively throughout the genome. Whether these enhancer-derived RNA (eRNAs) are merely noise, by-products of enhancer activity, or directly contribute to enhancer activity is not known. Through studying transcription factor Rev-Erb, we present evidences that eRNAs directly contributes to enhancer activity. Rev-Erbs are nuclear receptors that regulate gene expression involved in the control of circadian rhythm, metabolism, and inflammatory responses. By mapping genomic-wide binding of Rev-Erb using Chromatin-Immunoprecipitation follow by sequencing (ChIP-seq), we demonstrated that Rev-Erbs function as transcriptional repressors by binding to enhancers or promoters of target genes. Remarkably, the repressive functions of Rev-Erbs are associated with their ability to inhibit the transcription of eRNAs. To test whether eRNA are functional, we knockdown eRNAs using RNA interference (RNAi) or RNAseH-dependent RNA degradation pathway at two Rev-Erb regulated enhancers. Both approaches resulted in reduced expression of nearby mRNAs, implying a direct role of these eRNAs in enhancer function. By precisely defining eRNA start sites using a method that quantifies 5' ends of nascent RNA, we show that transfer of full enhancer activity to a target promoter requires both the sequences mediating transcription factor binding and the sequences encoding the eRNA transcript. Together, these studies provide evidence for direct roles of eRNAs in contributing to enhancer functions. Furthermore, we demonstrated that a Rev-Erb bound enhancer found in macrophages display circadian transcriptional activity in a cell type specific fashion. This further implicates the clinical and biological relevance of enhancer elements in context of genetic variance to population diversity and disease. This study also raised the possibility of targeting eRNA as a potential therapeutic approach.

106

"NUT" Midline Carcinoma of the Pelvis Masquerading as Ewing Sarcoma: A Case Report of This Rare and Deadly Disease

***J Lee*, T Kuhta, P Kent, J Cameron**

Rush University Medical Center, Chicago, IL

Background: Nuclear Testes Gene "NUT" midline carcinoma (NMC) is a rare, lethal epithelial cancer with a characteristic translocation, t(15;19) (q13;p13.1), involving the NUT gene on chromosome 15q14 fused with BRD4 on chromosome 9. It is a poorly differentiated, aggressive carcinoma, with metastases present at diagnosis in 50% of cases. It lacks effective treatment with a median survival time of 6.7 months. **Case Report:** The patient is an 18-year-old African American female who presented with a three-year history of worsening right hip pain and sciatica. CT showed a 13.5 x 8.7 x 12.4 cm right-sided pelvic bone mass with lateral displacement of uterus, colon, and bladder without metastases.

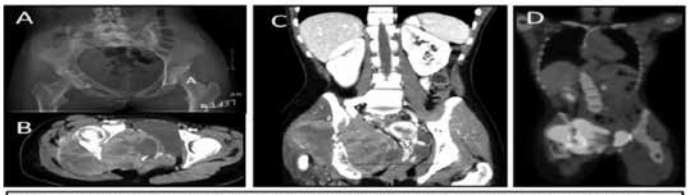


Figure 1: Pelvic Xray demonstrates lytic lesion in right ilium at the acetabulum (A). Large heterogeneous soft tissue mass centered in the right ilium with large soft tissue component surrounding it. There is extension deeper into the pelvis causing mass effect on the pelvic structures. (B,C) Large right pelvic mass centered around right ilium is markedly FDG avid on PET/CT.

The tumor pathology was originally thought to be Ewing sarcoma, however, Ewing is rare in African Americans and she lacked the characteristic EWSR1 gene rearrangement. Her immunochemistry showed reactivity to NUT protein, which has 100% specificity and 87% sensitivity, leading to a diagnosis of NMC. **Methods/Results:** We reviewed the literature on NMC and found 63 cases since 1990, with the majority occurring in the midline of head, neck and thorax and only 1 case involving the iliac bone. **Conclusions:** NMC arising in non-midline bone structures is an extremely rare event. The lack of knowledge about this rare disease and its non-diagnostic pathology often leads to misdiagnosis as squamous cell carcinoma, Ewing sarcoma or sinonasal undifferentiated carcinoma. Few labs have the NUT antibody for those pathologists who are aware of this entity. It is imperative that physicians have clinical suspicion for NMC in patients with poorly differentiated neoplasms and those that mimic Ewing sarcoma.

Poster Abstracts

107

Flexible Needles for Intracerebral Navigation

CA Lehocky, **CN Riviere**
Carnegie Mellon University, Pittsburgh, PA

Deep needle insertion into the brain is important for both intervention and diagnostics. Biopsy, deep brain stimulation, and delivery of chemotherapeutic agents to areas such as the brain stem require navigation from the cortical surface. Accurate needle placement is paramount to the effectiveness of these procedures. Traditional needles must follow straight trajectories as they are inserted into tissue, which can cause damage to critical anatomical structures and vasculature along the path. Also, any adjustment of final needle position requires complete removal of the needle and reinsertion from the cortical surface, which can lead to additional tissue damage. We have developed a method of robotically steering flexible needles within tissue, allowing for nonlinear trajectories that can improve targeting accuracy while also increasing safety. Thin, bevel-tipped needles curve naturally when inserted into tissue, due to the asymmetric force distribution upon the bevel tip. We have expanded upon this phenomenon to develop a robotically-controlled bevel-tipped flexible needle. Through the use of two independent motors, one for insertion and one for rotation, the needle can be commanded to change the direction of curvature. By developing a method of "duty-cycled rotation", we can also control the amount of needle curvature. Combining these two degrees of control (direction and amount of curvature), the needle can be made to follow predetermined three-dimensional curvilinear paths in tissue. This presentation will consist of an overview of the several areas of research undertaken in order to develop this clinical technology. The first area is the closed-loop control algorithm, which plans several feasible paths in 3D space that reach the target while avoiding obstacles, actuates the needle along the path, and updates the control scheme through imaging feedback. The second emphasis is on the design of the needle for effectiveness and safety. Finite element method simulations were conducted of needle insertions and rotations into a brain tissue model. While conforming to established safety limits of brain stress and strain, these simulations allowed for the development of a custom needle tip and determined the parameters of optimal needle diameter, bevel angle, and material. Finally, the results of flexible needle steering effectiveness and safety will be presented, from both *in vitro* simulations (gelatin model), as well as in live porcine brain.

109

Women in Science and Medicine Association: An Overview of Student Implemented Professional Development Programming at the University of Pittsburgh

A Liu, **J Boiko, A Marsh, R Gordon**
University of Pittsburgh School of Medicine, Pittsburgh, PA

Physician scientists in training are privy to unique training challenges as they strive to integrate didactic, laboratory, and clinical learning. The Women in Science and Medicine Association (WSMA) at the University of Pittsburgh School of Medicine is a professional organization dedicated to three core goals: I) Provide educational and supportive resources to female physician-scientists in all stages of training, II) Recruit students to pursue a physician-scientist career path and retain current trainees already committed to this path, and III) Empower advocates to aid and support female physician-scientists to obtain leadership roles and their career goals. To this end, we organize an annual seminar and workshop series addressing topics such as non-traditional careers, work-life balance as a physician scientist, and pursuing research opportunities during medical school. Past seminars have discussed salary negotiation strategies, starting a first lab, and balancing dual academic medicine and research careers. During each academic year, our programming consists of workshops and seminars, journal club meetings, lunch events with invited speakers. We also organize Doctoral Directions, a two-day regional conference discussing the training paths of and challenges faced by scientists in academia. This conference features speakers and panelists from the University of Pittsburgh and other nationally renowned research institutions and attracts the attendance of PhD, MD, and MD/PhD trainees. In addition to the traditional speaker or panel-discussion oriented seminars, we hope to incorporate more skills based workshops into our annual programming. For example, one past event, "Communication for Physicians and Scientists: How oral storytelling techniques can inform the way we talk about complex scientific ideas," was comprised of a two hour interactive session led by the student run organization Public Communication for Researchers. We are currently working to improve our professional development programming using pre- and post-event formal feedback to gauge student interest and to develop data metrics by which we can measure the utility of the professional development opportunities that our organization offers.

Poster Abstracts

110

Effect of Antibiotic Treatment on the Microbiota, the Developing Immune System, and Type 1 Diabetes Development in Non-Obese Diabetic Mice

***AE Livanos*[*], VE Ruiz[*], H Li[*], T DiLorenzo[†], and MJ Blaser[*]**
[*]New York University School Medicine, New York, NY; [†]Albert Einstein College of Medicine, New York, NY

The incidence of multiple autoimmune diseases including type 1 diabetes (T1D) has increased rapidly since 1950, which suggests that environmental factors associated with modern practices such as antibiotic use could modulate disease risk. In the non-obese diabetic (NOD) mouse model of T1D, disease incidence is higher in specific pathogen-free than in conventional facilities, reinforcing the importance of microbes. Another characteristic of NOD mice is that females have higher T1D incidence compared to males. We hypothesize that early-life antibiotic use alters gut microbiota essential for immune development, and promotes T1D development. We compared male and female control NOD mice to mice exposed to pulsed antibiotic treatment (PAT), which consists of 3 therapeutic pulses of Tylosin (macrolide) early-in-life, mimicking repeated childhood antibiotic exposures. T1D incidence was significantly increased in PAT males (53%) compared to control males (26%) by 31w ($p=0.04$). Sequencing of the V4 region of the 16S rRNA gene was performed to determine the effect of PAT on the gut microbiota. Phylogenic diversity was lower in PAT-treated male and female fecal samples at 3-, 6-, 10-, and 13-weeks of age as well as in cecal and ileal samples collected at 6-weeks of age. PAT also significantly altered beta-diversity at all time-points examined. *Bifidobacterium* species were found to be significantly higher in males, which are relatively protected from diabetes, compared to females as well as in control males compared to PAT-treated mice. Flow cytometry of small intestinal lamina propria (SI-LP) cells in pre-diabetic control mice showed that males had higher percentages of CD4+RORγT+(Th17) ($p=0.01$) and CD4+FOXP3+(Treg)($p<0.01$) cells than females. In males, PAT reduced the percent of SI-LP Th17 ($p<0.01$) and Treg cells ($p<0.01$); with no similar effect in females. Th17 cell percent was lower in males than in females in PAT ($p<0.001$). The proportions of splenic CD4 and CD8 T-cells producing IFNγ was increased in PAT males compared to controls specifically at 7-weeks of age ($p<0.01$ for both). These findings provide evidence that early-life antibiotic treatment alters the gut microbiota and T-cell populations both locally and systemically, and accelerates T1D onset.

111

Modulation of Interleukins in Sepsis Associated Coagulopathy: Interplay with Thrombosis

***Chris Low,* Danyeal Syed, Dan Khan, Sermin Tetik, Debra Hoppensteadt, Michael Mosier, Jawed Fareed**
Loyola University Chicago, Stritch School of Medicine, Maywood, IL

Introduction: Interleukins form a group of over 36 cytokines that have various origins, target receptors and functions. Sepsis syndrome is capable of activating the synthesis of interleukins as a defense response or inflammatory reaction to the pathophysiologic insult. The interleukins also directly or indirectly affect some of the regulators of hemostasis including tissue factor. Assessment of interleukins provides additional insight into the mechanisms involved in the pathogenesis of Sepsis Associated Coagulopathy (SAC). To understand the role of interleukins in the pathogenesis of SAC, it was hypothesized that in sepsis, the interleukins may be upregulated leading to hemostatic imbalance by generating thrombogenic mediators. **Materials and Methods:** Plasma samples (n=100) from SAC were retrospectively collected from various centers under approved IRBs with a defined criterion. Normal human pooled plasma (NHP) represented a pooled plasma collected from 10 healthy male and female volunteers. A Randox Technology biochip array was used to quantify IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8 and IL-10. Microparticles, microparticle associated tissue factor, D-dimer and tissue factor antigen were also quantified using ELISA methods. **Results:** The results for each parameter were compiled in terms of the Mean +/- SD, Mean +/- SEM, Median values and fold increase in comparison to the NHP. Compared to NHP, all of the interleukins were markedly higher in the SAC group. A wide scatter in all parameters was noted. In the inflammatory markers, the most pronounced increase was observed in IL-6 and IL-8 while IL-1β, IL-2 and IL-4 exhibited modest increase. In the thrombogenic markers, Tissue Factor and D-Dimer showed a measurable increase. D-Dimer, an indicator of fibrinolysis shows the most prominent increase. **Discussion:** This data suggests that there is a complex interplay between various cells and mediator-based mechanisms which contribute to the pathogenesis of SAC. Most notably, a dramatic increase ranging from 1000 fold to 100 fold were noted in IL-6, IL-8 and IL-10, while D-Dimer levels were also significantly increased by 18 fold. The simultaneous activation of thrombotic markers such as microparticles and tissue factor is highly suggestive of an interplay between interleukins and thrombogenesis in SAC. A profiling of these markers not only provides an understanding of mechanisms involved in SAC but may help in the risk stratification of these patients. Furthermore potential link between inflammation and thrombogenesis may lead to the development of anti-inflammatory and anti-coagulant drugs with dual sites of action.

112

Perk-Mediated ER Stress: Linking Acute Blast-Induced Neurotrauma with Tau-Dependent Neurodegeneration

***B.P. Lucke-Wold*[*,†], R.C. Turner[*,†], A.F. Logsdon[†], C.L. Rosen[*,†]**
[*]Department of Neurosurgery, West Virginia University, School of Medicine, Morgantown, WV; [†] The Center for Neuroscience, West Virginia University, School of Medicine, Morgantown, WV

Blast-induced traumatic brain injury (bTBI) is the 'hallmark injury' of modern warfare with up to 20% of U.S. service personnel being exposed. Despite the large clinical prevalence, the pathogenesis of neural injury after exposure to bTBI is poorly understood. Specifically, how blast-induced neurotrauma leads to an increased risk for development of neurodegenerative disease such as chronic traumatic encephalopathy (CTE) is unclear. Using a novel shock tube model of blast injury designed to simulate clinically relevant blast wave parameters we sought to illuminate the underlying mechanism of injury. Endoplasmic Reticulum (ER) stress has been implicated in both preclinical and clinical studies of neural injury. It

has not, however, previously been investigated in neurotrauma. In this work we seek to elucidate the contribution of PERK-mediated ER stress on neural injury over time in young adult male rats. The present study investigates acute post-injury time points (1.5, 3, 6, 12, and 24 h) in order to characterize the role of the PERK-mediated ER stress pathway on acute neural injury following moderate blast exposure. rtPCR data showed increased gene expression of ER stress marker *chop* ($F(6,21) = 16.01$, $p<0.001$) at 3 h. Protein analyses exhibited an increase in protein levels of CHOP at 24 h ($t = 3.838$, $p< 0.05$) and phosphorylation of eIF2α at 0.5h ($t=5.447$, $p<0.05$). Salubrinal, an ER stress modulator, reduced CHOP ($F(2,15) = 9.172$, $p<0.01$) and p-eIF2α ($F(2,15) = 5.145$, $p<0.05$) at 24h and 0.5h following blast exposure; respectively. Salubrinal also ameliorated increased impulsive behavior on the elevated plus maze ($F(3,20) = 7.510$, $p<0.01$). We examined cell type-specific effects and the role of PERK-mediated ER stress in tau phosphorylation events identified through co-localization studies with immunohistochemistry. Caspase12, a marker of apoptosis, was co-localized with CHOP in neurons (overlap coefficient, $r = 0.991$), but not in astrocytes (overlap coefficient, $r = 0.131$). AT270, a marker of tau hyperphosphorylation, was co-localized with the ER stress marker IRE1α in both human CTE brains (overlap coefficient, $r = 0.904$) and repetitive blast rat brains (overlap coefficient, $r = 0.807$). Elucidating the role that PERK-mediated ER stress plays in these events may lead to increased understanding of the mechanistic link between acute brain injury and chronic neurodegenerative diseases.

113

Deciphering Inflammation Using a Novel Activity Assay for Calcium-Independent Phospholipase $A_2\beta$

KR Malley, **O Koroleva, P Subramanian, S Korolev**

E.A. Doisy Department of Biochemistry and Molecular Biology, Saint Louis University School of Medicine, St. Louis, MO

The calcium-independent phospholipase $A_2\beta$ ($iPLA_2\beta$), which hydrolyzes phospholipids at the *sn-2* ester position, acts in the early stages of the inflammatory cascade through the production of arachidonic acid, a precursor of potent inflammatory mediators. Altered $iPLA_2\beta$ activity has been linked to multiple diseases such as myocardial ischemia, diabetes, and muscular dystrophy. In addition, mutations in the protein have been identified in patients with neurodegeneration such as Parkinson's disease and neuroaxonal dystrophy. $iPLA_2\beta$ activity is known to be regulated by multiple factors, such as calmodulin and ATP. However, the mechanisms by which $iPLA_2\beta$ is activated and regulated are poorly understood due to the lack of structural information. To gain insight into these mechanisms, basic enzymatic parameters such as the Michaelis constant K_m and catalytic rate constant k_{cat} are needed. Traditional assays using radiolabeled phospholipids present limitations to quantitative measurement of these parameters. We present a sensitive and convenient plate reader assay that monitors activity in real time using fluorescent phospholipid substrates. We show that $iPLA_2$ activity can follow Michaelis-Menten kinetics, and determine K_m and k_{cat} for a plasmenylcholine-based fluorescent substrate. This novel assay has multiple advantages over radioactive methods and can be performed in a high-throughput manner. The utility of the assay is demonstrated by measuring significant differences in enzyme activity in the presence of 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC)/substrate liposomes versus substrate alone. This assay will be essential in evaluating the effect of potential activators, inhibitors, or interacting proteins. It will also allow comprehensive characterization of disease mutants. Such an approach will elucidate basic structure-function relationships and provide critical insights for the development of novel inhibitors and modulators of $iPLA_2\beta$ activity to control inflammation.

114

Activation of Striatal D2-Receptors by Phasic and Tonic Dopamine Signals

PF Marcott**, **CP Ford

**Case Western Reserve University School of Medicine, Cleveland, OH*

The release of dopamine from axon terminals in the striatum plays a critical role in initiating and planning movement, processing rewards, and driving other goal-oriented behaviors. Dysregulation of the dopamine system is involved in a variety of psychiatric and neurological diseases, including Parkinson's disease, schizophrenia, attention-deficit and hyperactivity disorder, and drug addiction. Dopamine encodes behaviors on a fast, sub-second time scale. Other studies have measured rapid increases in extracellular dopamine in the striatum, however it remains unknown how this rapid rise in striatal dopamine leads to the activation of post-synaptic receptors. Since dopamine receptors on medium spiny neurons (MSNs) in the striatum do not couple to ion channels, receptor activation does not evoke a direct change in membrane conductance that can be measured with conventional approaches. This study uses viral overexpression of a G-protein coupled inwardly rectifying potassium (GIRK) channel in the striatum of mice. In this system, D2-receptors couple to GIRK channels and provide a rapid read-out of receptor activation on the millisecond timescale. Dopamine-mediated GIRK currents were recorded in striatal indirect-pathway MSNs in response to the bath application of a D2-receptor agonist. Evoked synaptic release of dopamine, by both electrical and optogenetic stimulation of dopamine terminals, produced robust D2-IPSCs. In addition, it was found that striatal D2-receptors demonstrated tonic activity in response to high ambient concentrations of dopamine. These results suggest that striatal D2-receptors can encode both rapid phasic dopamine transients as well as tonic dopamine signals.

Poster Abstracts

115

Psychosine Induces the Aggregation of Alpha-Synuclein in Krabbe Disease

***Michael S. Marshall*[1], Marta B. Santos[1], Benjamin R. Smith[1], Ludovico Cantuti-Castelvetri[1], Aurora Lopez-Rosas[1], Guanan Li[2], Richard van Breemen[2], Kumiko I. Claycomb[3], Jose I. Gallea[4], Soledad M. Celej[4], Stephen Crocker[3], Maria I. Givogri[1], and Ernesto R. Bongarzone[1]**

[1]Department of Anatomy and Cell Biology, College of Medicine and [2]Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois, University of Illinois Chicago, Chicago IL; [3]Department of Neuroscience, University of Connecticut Health Center, Farmington, CT; [4]Departamento de Quimica Biologica, CIQUIBIC, CONICET, Universidad Nacional de Cordoba, Cordoba, Argentina

Infantile Krabbe disease occurs due to a mutation of the lysosomal enzyme galactosylceramidase, resulting in the accumulation of unmetabolized galactosyl-sphingolipids (psychosine). This toxic build-up causes central and peripheral demyelination and neurodegeneration, along with cognitive and motor deficits, eventually leading to early death. Prompted by recent reports of misfolded protein inclusions found in other lysosomal storage diseases, we investigated the possibility of similar cellular inclusions in brain samples from Krabbe patients and twitcher mice, a pathological and enzymatic authentic murine model of infantile Krabbe disease. Thioflavin-S staining identified beta-sheet aggregated proteinaceous inclusions in both the twitcher and Krabbe brains, which was later confirmed by electron microscopy in the twitcher model. Thioflavin-S reactive inclusions were found to follow a regular spatial and temporal patterning throughout the twitcher life-span and be primarily located within neurons, and to a much lesser level, in microglia. In addition, inclusions were immunochemically determined to be composed of alpha-synuclein and ubiquitin, resembling Lewy bodies found in Parkinson's disease and other alpha-synucleinopathies. Immunoblotting of human tissue detected the presence of high molecular weight species of alpha-synuclein, providing further evidence of alpha-synuclein aggregation. Evidence for psychosine-induced aggregation of alpha-synuclein was suggested by the similar distribution of psychosine and synuclein aggregates in anatomical regions of the twitcher brain. Furthermore, in vitro assays determined that the rate of alpha-synuclein fibrilization was increased by the presence of psychosine in a dose-dependent manner. The identification of neuronal alpha-synuclein inclusions in Krabbe and twitcher brains, suggests that Krabbe disease be recognized as a new member of the alpha-synucleinopathies. In addition, these findings will hopefully introduce a new area of study in Krabbe disease and expand the therapeutic targets available to patients.

117

TDP-43 Regulates MicroRNAs, Linking Aging Related Diseases

Xiaowei Chen[*,1], Zhen Fan[*,1], *Warren McGee*[†,1], Mengmeng Chen[*,†,1], Ruirui Kong[*], Pushuai Wen[*], Xiaomin Chen[*], Jianghong Liu[*], Li Zhu[*], Runsheng Chen[*], Jane Y. Wu[*,†]

[*]IBP, CAS, China; [†]Department of Neurology, Center for Genetic Medicine, Lurie Cancer Center, Northwestern University School of Medicine, USA; [1]These authors contributed equally to this work

MicroRNAs play important roles in a wide range of biological processes. Aberrant regulation of miRNA genes contributes to human diseases, including various aging related disorders such as neurodegeneration and cancers. MicroRNA biogenesis is regulated by RNA binding proteins, including TAR DNA binding protein 43 (TDP-43), which is a DNA/RNA binding protein associated with both neurodegeneration and cancer. Here, we systematically examined miRNAs whose expression levels are regulated by TDP-43 using RNA-Seq coupled with siRNA-mediated knockdown approach. Candidate TDP-43 targeted miRNAs were validated by quantitative RT-PCR. Some of the TDP-43 regulated miRNAs appear to interact with TDP-43 directly. Alterations in isomiR patterns and miRNA arm selections following TDP-43 down-regulation indicated that TDP-43 plays a role in miRNA editing. We also examined the correlation of selected TDP-43 regulated miRNAs with their candidate target genes as well as their expression patterns in human cancers and neurodegenerative disorders. We examined the sets of genes targeted by each candidate miRNA, curated from both validation experiments and predictions from multiple available tools. Using a gene set enrichment analysis approach with different datasets, we are constructing the targetomes for TDP-43-regulated miRNAs and examining the association between TDP-43 regulated miRNA targets and biological processes or disease phenotypes. Our experiments have begun to validate the computational predictions made by this approach. For example, our data show that TDP-43 promotes lung cancer cell migration by regulating expression of TDP-43 targeted miRNAs. Our experiments have also begun to reveal previously unknown miRNA-target gene pairs that may play important roles in cancer and other diseases.

Poster Abstracts

118

Derangement in Lipolytic and Bile Acid Pathways May Provide a Link between Inflammation and Cardiometabolic Diseases

***N. N. Mehta*[1], S. Rose[1], E. Weiner[1], J. Doveikis[1], P. Krishnamoorthy[2], M. P. Playford[1], J.M. Gelfand[2]**

[1]National Heart Lung and Blood Institute, Bethesda, MD; [2]Englewood Hospital, Englewood, NJ; [3]University of Pennsylvania, Philadelphia, PA

Psoriasis is a common,chronic inflammatoryT-cell disease of the skin.Psoriasis is risk factor for cardiometabolic disease (CMD) beyond traditional risk factorsand provides an *in vivo* model to understand the potential links between inflammation and CMD in humans. The goal of this study was to interrogate biochemical profiles in plasma from psoriatic patients and healthy controls with the aim of characterizing metabolic changes observed in the context of inflammatory diseaseto better understand potential links between systemic inflammation and CMD. We enrolled patients with varying severities of psoriasis (n=90) and compared plasma from these patients to controls recruited from the same institution (n=30). The extracted samples were split into equal parts for analysis on the GC/MS and Orbitrap Elite accurate mass LC/MS/MS platforms (Metabolon, North Carolina, USA).Results were adjusted for age, sex, and body mass index. Psoriasis patients had similar age, gender and body mass index characteristics compared to those without psoriasis. We found increased lipolysis and β-oxidation of fatty acids in the psoriasis group. One of the most striking and consistent changes involved the accumulation of various essential, medium-chain, and long-chain fatty acids in plasma from patients with psoriasis ($p<0.001$ correcting for false discovery rate). The related elevation in glycerol, a marker of lipolysis, indicated increased release of free fatty acids from storage depots such as adipose tissue andthe skin. Accumulation of several acylcarnitines (laurylcarnitine, myristoylcarnitine, and palmitoylcarnitine) in plasma from psoriatic patients provided further evidence of altered lipid metabolism as conjugation of free fatty acids with carnitine is necessary for transport of these lipids into the mitochondrial matrix for subsequent β-oxidation. We also observed a reduction in plasma bile acids in patients with psoriasis, which may have important implications for other aspects of lipid metabolism and homeostasis. For example, trending ($0.05<p<0.10$) or significant ($p\leq0.05$) reductions in the conjugated primary bile acids glycocholate and glycochenodeoxycholate and secondary bile acids taurodeoxycholate, glycodeoxycholate, glycolithocholate sulfate, and glycoursodeoxycholate were noted in patients with psoriasis. In conclusion, plasma samples from patients with psoriasis exhibited profound metabolic perturbations among pathways which have established roles in both metabolic and cardiovascular diseases. Targeted pathway analyses will further elucidate potential mechanisms of these links.

119

Combined Deletion of SR-BI and CD36 Accelerates Diet-Induced Murine Atherosclerosis

L Cai, *JM Meyer,* A Ji, DR van der Westhuyzen

University of Kentucky College of Medicine

Background: Scavenger Receptor Class B Type I (SR-BI) and CD36 are members of the Class B Scavenger Receptor protein family that play important roles in lipid and lipoprotein metabolism, inflammation and immunity. SR-BI expression has been demonstrated to protect against the development of atherosclerosis in hypercholesterolemic mice, while conflicting data exist regarding the function of CD36 in atherosclerosis. The purpose of the current investigation was to determine whether combined genetic deletion of these two related proteins in mice would modulate a shared pathway leading to alterations in atherogenesis. **Methods:** WT, SR-BI -/-, CD36 -/- and SR-BI/CD36 double-deficient (DKO) mice were fed an atherogenic (Paigen) diet for 16 weeks. Plasma lipid profiles were determined by FPLC at 3 weeks and 6 weeks of feeding, and aorta and other tissues were collected for analysis at the end of the study. Atherosclerosis was quantified by enface analysis as the percent lesion area of the aorta. In a separate study, lethally irradiated DKO mice received bone marrow from WT, SR-BI -/-, CD36 -/- and DKO mice, and then fed for 16 weeks on the atherogenic diet. Bone marrow-derived macrophages (BMMs) and mouse peritoneal macrophages (MPMs) were treated with human HDL, LDL and acetylated or oxidized LDL to assess macrophage lipoprotein uptake and cholesterol efflux. **Results:** During atherogenic diet feeding, SR-BI -/- and DKO mice had similarly elevated serum total cholesterol (TC) levels (>3-fold) compared to WT and CD36 -/- mice. Atherosclerosis was enhanced in DKO mice by approximately by 3-fold compared to SR-BI -/- mice, while no lesions were detected in WT and CD36 -/- mice. There were no differences in atherosclerosis between irradiated DKO mice that received WT, SR-BI -/- , CD36 -/- or DKO bone marrow. Isolated BMMs and MPMs did not exhibit differences in lipoprotein uptake or foam cell formation. **Conclusions:** Combined deletion of SR-BI and CD36 accelerates atherosclerosis in atherogenic diet-fed mice, indicating a potentially synergistic protective function of these two proteins. Studies using bone marrow transplantation and isolated cultured macrophages suggest that this effect is most likely not mediated by bone marrow-derived cells including macrophages.

Poster Abstracts

121

Inhibition of Fibrinolysis Attenuates Influenza A-induced Lung Injury

L Morales-Nebreda, **M Eren, S Soberanes, R Nigdelioglu, M Rogel, M Chi, T Cho, DE Vaughan, GRS Budinger and GM Mutlu**
Northwestern University, Chicago, IL

Rationale: Influenza A is the leading cause of death from an infectious cause and ranks 8th in the list of attributable annual mortality in the United States. Currently available antiviral treatments are limited and may become ineffective as resistant strains emerge. Therefore, there is urgent need for alternative therapies. The coexistence of hemostatic alterations with inflammatory responses to viral infections supports the idea that common molecular mechanisms contribute to the regulation of these systems. Previous studies demonstrated that influenza A virus (IAV) induces the activation of both coagulation and fibrinolytic pathways, and that the impairment of the latter is mainly attributable to high levels of plasminogen activator inhibitor type I (PAI-1), the major inhibitor of tissue-type plasminogen activator (t-PA). Interferon-α (IFN-α) plays a key role in the control of viral replication and overall shaping of the immune response. We sought to determine whether the serine protease inhibitor PAI-1, plays a role in the immune response and morbidity and mortality associated with influenza A infection. **Methods**: Wild-type (C57BL/6J), *PAI-1*$^{-/-}$, *t-PA*$^{-/-}$ and *PAI-1* stab (transgenic mice expressing an active form of human PAI-1) mice were treated intratracheally with influenza A virus (A/WSN/33 [H1N1] 500 pfu/mouse in 50µl PBS). We assessed the levels of IFN-α in bronchoalveolar lavage fluid (BALF) on day 2 and 4, and influenza A-induced morbidity (weight loss) and mortality. **Results:** Compared with wild-type mice, *PAI-1*$^{-/-}$ mice had increased influenza A-induced morbidity and mortality. In contrast, both *PAI-1 stab* and *t-PA*$^{-/-}$ mice had improved survival. In wild-type mice, IAV induced a significant elevation IFN-α levels in BALF. *PAI-1 stab* mice had a 2-fold increase, while *PAI-1*$^{-/-}$ mice had a 69% reduction in IFN-α levels in BALF compared to wild-type mice. **Conclusions:** Inhibition of fibrinolysis by means of genetic overexpression of *PAI-1* or deletion of *t-PA* augments the antiviral response and attenuates the morbidity and mortality associated with influenza A infection. These results suggest an important functional role for PAI-1 and fibrinolysis in the pathogenesis of influenza A infection. Modulation of the fibrinolytic pathway or PAI-1 may potentially be useful as a target for novel therapeutics in the management of influenza A-induced lung injury.

122

Alpha Glutathione-S-Transferase: A New Bio-Marker for Liver Injury?

***I.M. Mwangi*[1], J.A. Balko[1], W.M. Lee[1], F.H. Wians[2], M. Poirier[3]**
[1]Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX; [2]LoneStar Pathology Laboratory and Scientific Writing Consultants, Helotes, TX; [3]Qualigen, Inc., Carlsbad, CA

Background: Serum alanine and aspartate aminotransferases (AST/ALT) have been the gold standard for detection and quantitation of liver injury for over six decades, but have relatively long half-lives (t1/2) (literature estimates ~17 and 47 hrs, respectively) and thus do not reflect immediate changes in liver injury or recovery. A new point-of- care immunoassay for α-glutathione S-transferase (α−GST) measures this cytosolic liver enzyme with a predicted t1/2 of 60-90 minutes based on preliminary studies and might enable earlier detection of improving or worsening liver injury than conventional enzyme testing. **Methods:** Sera collected daily from 31 patients enrolled in the Acute Liver Failure Study Group, with acetaminophen toxicity (APAP), drug-induced liver injury (DILI), ischemic hepatopathy, or autoimmune hepatitis were analyzed to determine α−GST t1/2 and compared to the t1/2 for AST and ALT in these same patients. α−GST values were determined using the Qualigen FastPack® (Carlsbad, CA), a chemiluminescent immunoassay utilizing a paramagnetic particle matrix with an upper limit of normal (ULN) of 11 ng/ mL. AST and ALT ULN = 40 IU/L. The t1/2 for α−GST, AST and ALT were calculated from the peak value using an exponential trend line equation of the serial values. **Results:** Median α−GST for all etiologies were elevated on Day 1, returning to normal by Day 3. However, median AST and ALT values did not reach normal even at Day 7. The median t1/2s for α−GST, AST, and ALT were: 6.4, 22.2, and 33.9 hours, respectively. **Conclusion:** α−GST is a more responsive marker of liver injury/recovery, allowing for real time assessment of improvement or worsening of liver disease.

123

Controlling the Intracellular Delivery of Spherical Nucleic Acids by Engineering Oligonucleotide Sequence

S Narayan, **C Choi, L Hao, C Calabrese, E Auyeung, C Mirkin**
Northwestern University, Evanston, IL

Intracellular delivery of nucleic acids for gene regulation typically requires the use of cationic carriers, but cytotoxicity associated with such carriers reduces their attractiveness for therapeutic use. Spherical nucleic acids (SNAs), consisting of densely packed, highly oriented oligonucleotide strands attached to the surface of nanoparticles, are able to overcome the typical challenges of nucleic acid delivery. SNAs have been shown to effectively enter 50 different cell types without the use of auxiliary transfection agents and exhibit minimal cytotoxicity. Recently, the mechanism of endocytosis of these polyanionic structures was shown to be dependent on class A scavenger receptors, which allow for uptake of SNAs into cells through lipid raft-mediated endocytosis. Linear poly(guanosine) DNA strands are natural ligands for class A scavenger receptors, whereas DNA strands composed of adenosine (A), thymidine (T), or cytosine (C) are

non-ligands. We expect that exploiting the natural interactions of class A scavenger receptors with poly(guanosine) oligonucleotide strands, by constructing SNAs whose constituent oligonucleotide strands are rich in guanosine (G), will maximize the uptake of SNAs into cells. In this work, we show that G-rich SNAs exhibit several-fold higher uptake into cells than SNAs rich in A, T, and C. The phenomenon of increased intracellular delivery can be explained, at least partly, by an increased affinity of class A scavenger receptors for G-rich SNAs; measurements of binding affinity show that G-rich SNAs exhibit stronger binding to class A scavenger receptors than SNAs rich in A, T, and C. This work presents an effective strategy to maximize the intracellular delivery of SNAs, which is potentially applicable to other nanoparticle systems, thus establishing an important design consideration for nanoparticle-based intracellular delivery of therapeutics.

124

Characterizing the Role of Intestinal Stem Cells During Regeneration in a Mouse Model of Colitis

R Nattiv, **H Tian, Y Nusse, OD Klein**

Department of Pediatrics, UCSF, San Francisco, CA; Department of Orofacial Sciences, UCSF, San Francisco, CA

Background: The intestinal epithelium is comprised of a single layer of cells that lines the gastrointestinal tract and is continuously replaced during homeostasis. The role of the intestinal stem cells and mixed progenitors during regeneration following acute injury is not fully understood. **Methods:** *Lgr5-EGFP*-IRES-CreERT2; *Rosa26-RFP* and *Bmi1*-CreER; *Rosa26-RFP* mice were fed 3% dextran sodium sulfate (DSS) in drinking water for five days *ad libitum*. Subsequently mice were fed regular drinking water. Mice were injected with tamoxifen at discrete time points to induce Cre activity and sacrificed at several time points to assess the presence of *Lgr5*- and *Bmi1*-expressing cells and determine the ability of these cells to generate progeny. Ki67 and Edu staining was performed to monitor for proliferation. **Results**: Following DSS-induced injury, mice displayed characteristic signs of colitis, including weight loss, diarrhea, and bloody stool. Histologic examination revealed ongoing proliferation in the colonic crypt throughout the recovery period. Interestingly, *Lgr5*-expressing crypt base columnar cells (CBCs) were absent following injury and during early recovery (days 1-2). *Lgr5*-expressing CBCs gradually repopulated and cell counts returned to normal by day 4-6 of recovery. Similarly, *Bmi1* expression was also absent during injury and early recovery. In contrast, we identified a relative increase in the number of secretory progenitor cells during early recovery. These cells were marked by the expression of *Atoh1* and were found to be proliferating. **Conclusion:** *Atoh1*-expressing secretory progenitors may evade DSS-induced intestinal injury and contribute to the early regenerative process. Future experiments will focus on the mechanisms that allow *Atoh1*-expressing cells to evade DSS-induced intestinal damage and on a possible role for reversion of these secretory progenitors through a dedifferentiation process during early recovery.

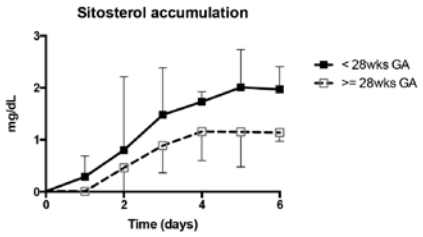
125

Kinetics of Plant Sterol Metabolism in Neonates Receiving Intralipid

TH Nghiem-Rao*, **A Mavis****, **EM Polzin**†**, MF Firary**†**, PM Simpson****, **SB. Patel****#

**Medical College of Wisconsin, Milwaukee, WI; †Children's Hospital of Wisconsin, Milwaukee, WI; #Zablocki Veterans Affairs Medical Center, Milwaukee, WI*

Background: Total parenteral nutrition (TPN) is essential for the survival of critically ill newborn infants. However, prolonged exposure leads to PN-associated liver disease (PNALD) particularly in preterm infants. Plant-derived sterols in Intralipid (IL) likely contribute to liver injury. Data regarding plant sterol metabolism in infants during IL infusion are lacking.



Objective: To determine the kinetics of plasma plant sterol concentrations in neonates receiving IL. We hypothesize that plasma plant sterol concentrations are related to IL exposure and gestational age (GA) affects plant sterol metabolism. **Design/ Methods:** Serial measurements of sitosterol, campesterol, and stigmasterol were performed on infants receiving IL for at least 5 days by GC/MS. Average area under the curve over the first 5 days of IL (AUC5) was calculated and random coefficient modeling was used to determine steady-state concentration (C_{ss}) and time to reach half of steady state ($T_{1/2}$). The decay constant (λ) was modeled using an exponential decay curve. **Results:** 36 infants (median (IQR)), GA (32.4 weeks (28.6-36)) and birth weight (1.46 kg (1.07-2.32)) were studied. Plasma sitosterol and campesterol increased to reach median C_{ss} of 1.33 mg/dL and 1.08 mg/dL, respectively. The $T_{1/2}$ of sitosterol was 1.8 days and 0.9 days for campesterol. Levels of stigmasterol remained near 0.01 mg/dL. AUC5 showed that sterol exposure was highest for sitosterol (0.74 mg/dL x day), followed by campesterol (0.72 mg/dL x day) and stigmasterol (0.01 mg/dL x day). Most infants fit an overall elimination model with λ=0.09 for sitosterol and 0.026 for campesterol. Infants born <28 weeks GA had significantly higher sitosterol AUC5 (p=0.032) and shorter sitosterol $T_{1/2}$ (p=0.019) than infants born $\geq$28 weeks GA. Differences in sitosterol AUC5 between GA groups remained significant after adjusting for cumulative sterol dose, sex, and race (p=0.012) (Figure). **Conclusions:** This is the first study to demonstrate kinetics of plant sterols in infants receiving TPN. Plant sterols accumulate rapidly in neonates during IL infusions, but elimination occurs slowly over weeks. Preterm infants receiving IL have higher exposure to plant sterols, suggesting that they may have poorly developed mechanisms of metabolizing plant sterols. These findings may provide a mechanistic insight into the vulnerability of preterm infants to this devastating complication.

Poster Abstracts

126

Cognitive Function and Delayed Neuronal Death in Swine Following Cardiac Arrest and Resuscitation

Anh Nguyen[1], **Brandon Cherry[1], Myoung-Gwi Ryou[2], Arthur Williams[1], Roger Hollrah[1], Charla Baker[3], Gourav Roy Choudhury[2], Albert H. Olivencia-Yurvati[3], Robert T. Mallet[1,3]**
Departments of [1]Integrative Physiology and Anatomy, [2]Pharmacology and Neuroscience and [3]Surgery, Univ. North Texas Health Science Ctr., Fort Worth, TX

Cardiac arrest, a leading cause of death in the U.S., kills >90% of its victims and survivors often are disabled by permanent brain injury inflicted by ischemia-reperfusion. Neuronal death usually occurs 3-4 days after the acute insult, a phenomenon called delayed neuronal death. Cerebellar Purkinje cells, which coordinate involuntary motor signaling, and CA1 neurons of the hippocampus, which are involved in memory and cognition, are especially vulnerable to post-ischemic death. In a swine model of cardiac arrest, we are testing the hypothesis that pyruvate, a metabolic intermediate and antioxidant, depresses neuronal injury after cardiac arrest, thereby fostering neurobehavioral recovery. Yorkshire swine (30-40 kg) were subjected to cardiac arrest-resuscitation or non-arrest sham protocols. Ventricular fibrillation was induced by electrical pacing. Precordial compressions (100/min) were given at 6-10 min arrest, and then sinus rhythm was restored with transthoracic countershocks. Sodium pyruvate infusion (0.1 mmol/kg/min *iv*) during CPR and the first 60 min after return of spontaneous circulation (ROSC), achieved arterial concentration of 3.5mM; controls received equivalent NaCl infusion. Neurological exams and cognitive tests were performed daily before and after cardiac arrest-resuscitation. The most severe neurological impairment was observed 24 h after cardiac arrest. Brain regions were fixed in 4% paraformaldehyde and H&E stained at 3d or 7d of ROSC. In the cerebellum, more than 70% of the Purkinje cells were shrunken, lacked dendrites and displayed condensed cytoplasm at 7 d ROSC; in contrast, in shams the majority of Purkinje cells retained the characteristic thick dendrites and well-defined nuclei. Thus, cardiac arrest-resuscitation produced marked changes in cognition and in Purkinje cell viability evident 7d after acute insult.
Support: NINDS 076975

127

Deep Brain Stimulation for Traumatic Brain Injury: Changes in Cognition and Function Associated with Changes in Relative Brain Activity

DM Nielson, **PB Sederberg, WJ Mysiw, MV Knopp, J Bogner, JD Corrigan, AR Rezai**
The Ohio State University, Columbus, OH

Over 1.7 million Americans sustain a traumatic brain injury (TBI) per year. Approximately 20% of these individuals sustain a moderate to severe TBI, commonly resulting in lifelong disability. Deep brain stimulation (DBS) has shown promising results for a number of psychiatric and neurological disorders such as obsessive-compulsive disorder and movement disorders. Here we report findings from an open label trial of DBS treatment in 4 subjects with severe disability who sustained a TBI more than 10 years prior. We demonstrate a relationship between changes in function and cognition following DBS and changes in relative brain metabolic activity. Following surgery and stimulation titration, participants received stimulation alone for 6 weeks, followed by 6 weeks of stimulation combined with rehabilitation therapy, and a 9-month follow up period. Brain positron emission tomography (PET) scans with 18F-fluorodeoxyglucose and a battery of cognitive and functional measures were collected over the course of the study. Statistical analysis was conducted with linear mixed effects regression to account for subject-level variance in the relationship between repeated PET measures and behavioral assessment. Performance on measures of function and cognition correlated with relative brain activity across subjects in several frontal regions, many of which also had significant decreases in relative pet activity over the course of the study. Combined, this indicates that the effects of DBS on cognition and adaptive function in TBI may be related to a decrease in relative activity in frontal regions.

128

Profibrotic Gene Expression by TGF-β Requires Mitochondrial ROS Generation

R Nigdelioglu, **S Soberanes, T Cho, L Morales-Nebreda, M Rogel, M Chi, NS Chandel, GM Mutlu and GRS Budinger**
Northwestern University, Chicago, IL

Background: Organ fibrosis is characterized by the accumulation of fibroblasts and the deposition of excessive extracellular matrix protein. Upon injury, Transforming Growth Factor β (TGFβ) is activated and binds to its receptors on fibroblasts to induce their proliferation and differentiation. Canonical TGFβ signaling involves the activation of small mothers against decapentaplegic (Smads), which bind to DNA to enhance the transcription of profibrotic genes. TGFβ also acts through Smad-independent pathways including p38, Akt, ERK1/2, which modulate target gene expression. We hypothesized that TGFβ induced generation of mitochondrial reactive oxygen species (ROS) is required for activation of downstream kinases to augment Akt and p38 phosphorylation.Methods: Normal human lung fibroblasts (NHLFs) were treated with TGFβ following 30 minute pretreatment with mitochondria-targeted antioxidant (Mito-Vit E) and control (TPP) and 61 kinase activation was screened using phosphokinase assay kit. NHLFs were also treated with TGF-β following 30 minute pretreatment with a superoxide dismutase/catalase mimetic (EUK134), Mito-Q or Mito-Vit E, TPP or no treatment and the expression of phosphorylated ERK1/2, Akt, p38 proteins were measured using immunoblotting. NHLFs were treated with TGF-β following 1 hour pretreatment with p38, Akt and ERK1/2 kinase inhibitors (SB203580, LY294002, UO126 respectively) and Smad-mediated genes N0X4, CTGF, SMA were measured 24 hours later using qRT-PCR. **Results:** Changes in p38 and Akt phosphorylation were found in phosphokinase screen assay and these changes were rescued by Mito-Vitamin E treatment. Akt and P38 phosphorylation were induced 10 minutes after TGFβ treatment and EUK-134 downregulated the phosphorylation of P38 and Akt. ERK1/2 phosphorylation was not induced 10 minutes after TGFβ treatment. The mRNA expression of TGFβ-induced Smad-mediated genes NOX4, CTGF, SMA were rescued following P38 and Akt kinase inhibitors (SB203580, LY294002). ERK1/2 kinase inhibition slightly increased TGFβ induced Smad-

mediated genes. **Conclusions:** Mitochondrial ROS are required for TGFβ-induced Akt and P38 activation and these ROS-induced Akt and P38 activation are required for Smad-mediated target gene expression following TGFβ binding with TGFβ type I receptor.

129

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

Novikov, O., **Parks, A., Rolfe, S., Narashiman, S., Stanford, E., Ubellacker, J., Sherr, D.**

Department of Environmental Health, Boston University School of Medicine, Boston, MA

This study focuses on the AhR, an environmental chemical receptor/transcription factor long associated with cancer. Historically, AhR activation by environmental ligands was seen to facilitate mutations through CYP1 up-regulation. Our findings suggested that the AhR plays potentially a more critical role in the later, lethal stages of cancer. This new concept represents a major paradigm shift and begs the question of what endogenous ligands drive AhR activity and tumor invasion in the absence of environmental AhR ligands (constitutive AhR activity). Our long-term goal is to identify endogenous ligands present in human mammary tumor cells, to determine how their production is controlled, and to assess how environmental AhR ligands alter this process. Previous studies provide evidence that tryptophan metabolites are potent endogenous AhR ligands. Our data demonstrate that four tryptophan metabolites, kynurenine (KYN), kynurenic acid (KA), xanthurenic acid (XA), and indoxyl sulfate (IS), are potent AhR agonists. We therefore hypothesize that: Tryptophan metabolites produced by KYN and IS pathways of tryptophan metabolism drive constitutive AhR activity and thereby enforce cell growth and/or invasion of malignant human mammary epithelial cells. As a corollary, we postulate that environmental AhR ligands distort this signaling. Treatment with metabolites of the IS and KYN pathway induced AhR activity and up-regulatd expression of AhR-driven genes in all mammary epithelial cell lines tested. Moreover IS disrupted normal colony formation of non-malignant mammary epithelial cells in Matrigel and lead to increased invasiveness of non-malignant cells in a trans-well assay. qPCR and western blot analysis revealed that that malignant mammary epithelial cell lines highly express TDO, a key enzyme required for the KYN pathway, while moderate to no expression of TDO was found in non-malignant cells. Reduction of *TDO* expression using a short-hairpin TDO-directed RNA resulted in reduction of *Cyp1B1*, a well-known transcriptional target of the AhR. In addition, down-regulation of *TDO* lead to reduction in expression of metalloproteinases which play a role in cell invasion, *MMP1, MMP9, MMP3*. Previous microarray data and promoter analysis suggests that expression of these metalloproteinases in mammary epithelial cell lines is driven by transcriptional activity of the AhR. We hypothesize that reduced expression AhR target genes after shRNA-induced TDO down-regulation is due to reduced production of endogenous AhR ligands via the kynurenine pathway of tryptophan metabolism. LC-MS analysis is currently being used to identify and quantify putative AhR ligands produced by tryptophan metabolism in malignant and non-malignant mammary epithelial cells.

130

The Transient Receptor Potential Vanilloid 4 Ion Channel Transduces Mechanical Loading in Cartilage

***CJ O'Conor*[*†]**, **HA Leddy[†]**, **HC Benefield[†]**, **WB Liedtke†**, **F Guilak[*†]**

[*]UNC-Chapel Hill, Chapel Hill, NC; [†]Duke University, Durham, NC

Introduction: Osteoarthritis (OA) is a degenerative joint disease affecting over 27M Americans. Mechanical factors play a key role in regulating the metabolic and functional properties of articular cartilage, and thus play an important role during OA pathogenesis. However, the mechanisms of chondrocyte mechanotransduction are poorly understood. TRPV4 (Transient Receptor Potential Vanilloid 4) is a Ca^{2+}-permeable ion channel expressed by articular chondrocytes that we propose to transduce mechanical loading in articular cartilage. Our goal was to elucidate the role of TRPV4-mediated Ca^{2+} signaling in response of chondrocytes to mechanical loading and uncover potential mechanisms for this phenomenon. **Methods:** Chondrocyte-laden agarose constructs underwent dynamic compressive loading +/- TRPV4 antagonist GSK205. Additional constructs were instead pulsed with GSK101, a TRPV4 agonist. Constructs were evaluated for gene expression changes, extracellular matrix accumulation, and mechanical properties. **Results:** GSK101 elicited intracellular Ca^{2+} transients in agarose-embedded chondrocytes, which were fully inhibited by GSK205 co-incubation. Dynamic loading enhanced chondrogenic (TGF-β3) and inhibited catabolic (ADAMTS5) gene expression, as well as enhanced matrix accumulation and mechanical properties of the constructs. Adding GSK205 during loading entirely blocked the effects of mechanical loading (GSK205 exposure alone had no measurable effects). Chemical activation of TRPV4 with GSK101 similarly enhanced TGF-β3 and inhibited ADAMTS5 expression, and also increased matrix content and mechanical properties of the constructs, analogous to the effects of mechanical loading. **Discussion:** Our results support our hypothesis that the chondroprotective response of chondrocytes to dynamic mechanical loading is mediated by TRPV4 channel activation. Furthermore, this regulation appears to involve a TRPV4-mediated induction of chondrogenic factors, such as TGF-β3, and suppression of catabolic factors, such as ADAMTS5. Further understanding of TRPV4-mediated cartilage mechanotransduction will hopefully lead to the development of novel disease-modifying pharmacotherapies for osteoarthritis, as well as improved tissue-engineering strategies for cartilage repair.

131

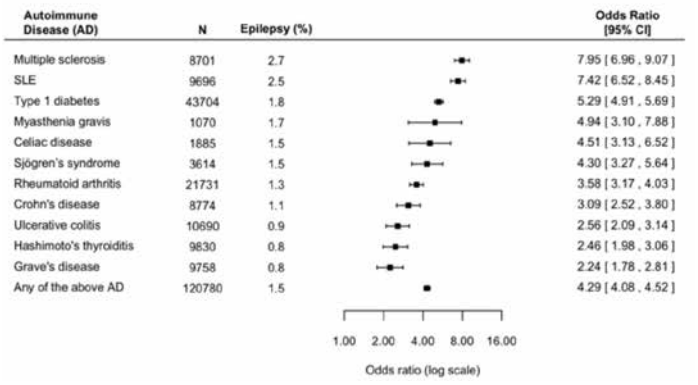
Population-Level Evidence for an Autoimmune Etiology of Epilepsy

***MS Ong*[1,2]**, **IS Kohane[2,3]**, **T Cai[4]**, **KD Mandl[2,3]**

[1]University of New South Wales, Sydney Australia; [2]Department of Pediatrics, Boston Children's Hospital, Boston MA; [3]Harvard Medical School, Boston MA; [4]Harvard School of Public Health, Boston MA

Background: Epilepsy is a debilitating condition, often with neither a known etiology nor an effective treatment. An autoimmune etiology has recently been hypothesized. **Objective:** To conduct a population-level study investigating the relationship

between epilepsy and common autoimmune diseases (AD). **Methods:** A retrospective study using claims from a nation-wide employer-provided health insurance plan in the US. Subjects were beneficiaries enrolled between 1999 and 2006 (n= 2,518,034). We assess the relationship between epilepsy and 11 AD, selected a priori: multiple sclerosis, systemic lupus erythematosus (SLE), type 1 diabetes, myasthenia gravis, celiac disease, Sjögren's syndrome, rheumatoid arthritis, crohn's disease, ulcerative colitis, Hashimito's thyroiditis, and Grave's disease. **Results:** The risk of epilepsy is significantly heightened among patients with AD (OR 4.3, 95% CI 4.1-4.5, p<0.0001). Elevated risk is consistently observed across all 11 AD (Figure 1). Exposure to aminosalicylates, anti-inflammatory biologics and non-steroidal anti-inflammatory drugs confers a protective effect.**Conclusion:** Our findings support the hypothesis of a common factor in the pathogenesis of autoimmune disorders and some forms of epilepsy, and shine new light on approaches to treating epilepsy. **Figure 1. Elevated risk of epilepsy in patients with AD, compared to those without AD. Results were adjusted by gender and age.**



132

From ST2 Omics to ST2 Therapeutics in Graft-Versus-Host Disease

J. Zhang*, A. Ramadan*, C. Mumaw*, I. Tawar†, E. Daguindau*, *S. Paczesny**

*Indiana University, Indianapolis, IN; †Mie University, Japan

Allogeneic hematopoietic stem cell transplantation (HSCT) is a potential curative therapy for cancers of the blood and bone marrow. However, graft-versus-host disease (GVHD) remains the major barrier to the success of HSCT, with mortality rates around 50%. Current strategies to suppress GVHD also compromise graft-versus-tumor (GVT) response. We have recently reported that elevated soluble suppression of tumoreginicity 2 (ST2) measured in plasma of post-HSCT patients predicted GVHD-related mortality (*NEJM*, 2013). ST2 is the newest member of the IL-1 receptor family whose sole known ligand is IL-33. Soluble ST2 acts as a decoy receptor for IL-33 and drives T_H2 cells toward a T_H1 phenotype. Thus, we hypothesized that blockade of the soluble ST2 will increase free IL-33 that will be able to bind to T cell, driving CD4+ T cell toward a T_H2 phenotype which will alleviate GVHD. First, we confirmed that levels of plasma ST2 were increased in multiple clinically relevant GVHD murine models. Anti-ST2 antibody given with a prophylactic schedule (before GVHD symptoms) significantly reduced GVHD severity (GVHD score, 2.31 ± 0.19 vs. 5.60 ± 0.50, P < 0.001) and mortality (70% vs. 40%, P < 0.05). Anti-ST2 treatment significantly increased plasma IL-33 and shifted T_H1/ T_H2 balance toward T_H2 (decreased T-bet, the T_H1-specific transcription factor, increased Gata-3, the T_H2-specific transcription factor, and their corresponding cytokines). Interestingly, ST2 blockade expanded CD4+Foxp3+ regulatory T cells. In addition, the membrane-bound form of ST2 is also expressed on a novel T_{Helper} subset, T_H9, which has both roles similar to T_H2 cells which suppress GVHD and antitumor activity superior to T_H1 cells in a melanoma model. Thus, we next hypothesized that ignition of the ST2/IL-33 pathway will suppress both fatal immunity and tumoreginicity via IL-9. We first modeled a donor-derived cellular therapy, and demonstrated that polarization of T_H9 cells with IL-33 enhanced ST2, PU.1 (T_H9-specific transcription factor) expression. Second, in a GVHD experimental model, IL-33-induced T_H9 adoptive transfer prevented GVHD better than T_H2 and IL4/TGFβ differentiated T_H9 cells by limiting the expansion of T_H1 in key target organs. IL-33-induced T_H9 adoptive transfer also increased GVT responses better than T_H1 cells and better than IL-4/TGFβ differentiated T_H9 by a granzyme-dependent mechanism. These data suggest that the ST2/IL-33 pathway could potentially enhance the curative potential of HSCT by increasing the antitumor effect and suppressing GVHD through modification of the imbalance between T_H1 and T_H2 - T_H9.

133

A Role for Histidine Rich Protein II in Cerebral Malaria Pathogenesis

P Pal**, B.P. Daniels*, R.S. Klein*, D.E. Goldberg*

*Washington University in St. Louis, MO

Cerebral malaria (CM) is the most severe manifestation of malaria and is characterized by decreased sensorium, coma, death, or lasting neurological complications in survivors. CM pathophysiology is thought to center along the vascular endothelium and is characterized by increased inflammatory cytokines, vascular leakage and a high parasite burden both in the blood stream and sequestered along microvascular walls. A breakdown of the complex protein network necessary to maintain the integrity of the blood brain barrier (BBB) has been observed on autopsy in patients infected with *P. falciparum*, the *only* species which causes human CM. Histidine rich protein II (HRPII) is a parasite protein which accumulates up to 100 µg/mL in the bloodstream of patients. It has also been seen lining microvascular endothelial walls in post-mortem analyses, and HRPII levels have been shown to correlate with disease severity. Using a blood brain barrier model, we observe a leakage in the presence of recombinant HRPII evidenced by an increase in permeability of human brain microvascular endothelial cell monolayers coupled with a decrease in their trans-endothelial electrical resistance. Longer exposures results in cell surface blebbing, and apoptosis. We can also observe this leakage in mice by following sodium fluorescein permeability into the brain parenchyma. We therefore propose that HRPII may act as a toxin and contribute to cerebral malaria by compromising the integrity of the blood brain barrier. We are following up on these findings using both cultured primary cells and a mouse model for cerebral malaria to identify the mechanism of action of HRPII.

Poster Abstracts

134

The Role of Cellular Senescence in Stem Cell Transplant Dysfunction

***Allyson Palmer*[1,2], Tamar Tchkonia[1], James Kirkland[1]**

[1]Robert and Arlene Kogod Center on Aging, The Mayo Clinic, Rochester, MN; [2]Medical Scientist Training Program, The Mayo Clinic, Rochester, MN

Adult stem cell transplantation is an emerging therapeutic strategy with wide potential to treat numerous age-related disorders such as cardiovascular disease, diabetes, and neurodegenerative disease. However, very little research has explored the causes of decreased efficacy of stem cell transplantation in the elderly population, who may have the most to gain from these new therapeutic strategies. Our laboratory is attacking this question using a 'seed vs. soil' model, asking whether an inherent dysfunction in the transplanted cells, the 'seed', or an inhospitable aged host microenvironment, the 'soil', is responsible for decreased therapeutic potential. We are specifically interested in the role of senescent cells in this interaction between 'seed' and 'soil' in transplantation. Senescent cells are growth-arrested cells that remain metabolically active and have been shown to promote a pro-inflammatory environment through the secretion of various cytokines, growth factors, and matrix remodeling factors, collectively known as the senescence-associated secretory phenotype (SASP). With aging, obesity, and in some disease states such as diabetes, senescent cell numbers increase throughout the body and are thought to contribute to tissue dysfunction. Therefore, the long-term goal of this study is to determine the role of increasing senescent cell burden in stem cell transplant failure. Our hypothesis is that removal of senescent cells from either the donor cells or the recipient mouse improves transplant outcomes. In these studies, we use a novel mouse model (INK-ATTAC) in which senescent cells can be selectively cleared from the mouse via apoptosis by administration of a transgene-targeting drug (AP20187) that does not affect normal cells. We studied the transplantation of adipose-derived stem cells on dorsal skin wounds in mice, allowing easy tracking of stem cell engraftment and incorporation into regenerating tissue, as well as wound closure. Senescent cells are cleared either from the recipient prior to transplantation of adipose-derived stem cells, to prepare the host environment (the 'soil') for transplant, or senescent cells are cleared from mice before isolation of stem cells, to prepare the transplanted cells (the 'seed'). Insights we gain into the role of senescent cells in stem cell transplantation may be hugely beneficial to the development of new therapeutic strategies that allow the application of emerging stem cell technologies to the elderly population.

135

The Role of Meal Replacements in Regulating the Hedonic Brain Network of Appetite

***Brielle Paolini,* Donald Williamson, Paul Laurienti, W. Jack Rejeski**

**Wake Forest Health Sciences, Winston-Salem, NC*

Background: Obesity has become a public health crisis, not sparing a quickly expanding population of older adults. Obesity is a major risk factor for chronic disease; thus, there is a growing need to understand the etiology of food consumption among older adults. Important to this research direction is the role that liquid meal replacements (MR) have in calorie-restricted diets for weight loss. We hypothesize that MR beverages modulate a hedonic brain network of appetite (HBN-A) and decrease state-craving. **Methods:** The sample included (n=14) obese, older adults. The participants completed two visits. During each visit, participants consumed a calorie-controlled breakfast. After a 2.5 hour fast, participants either consumed a can of BOOST ® (MR beverage) or an equivalent amount of water. On both visits, an MRI scan was performed and the Food Craving Questionnaire (FCQ_{state}) was administered. Functional brain networks were created for each individual. Hubs-of-interest for the HBN-A were defined as regions of interest with a greater number of connections in the fasting condition ($p<0.10$). To fully characterize the HBN-A, the direct and indirect connections among these hubs were quantified between conditions. **Results**: In investigating the HBN-A, we found that in the fasting state, brain networks had hubs in the following regions: anterior cingulate cortex (ACC), amygdala, insula, right hippocampus and superior temporal pole. Importantly, all interconnections among these five hubs were greater in the fasting condition than the MR condition. For direct connections, the effect sizes ranged from 0.38 to 0.74; whereas, for indirect connections, the effect sizes ranged from 0.53 to 0.82. We also show that when individuals consumed a MR beverage, they also reported significantly less craving. **Conclusions**: In summary, we found that MR beverages down-regulated the HBN-A after a short-term fast in older, obese adults. Additionally, the consumption of MR beverages also lowered craving, reinforcing their value in weight-management programs.

136

A Pilot Study of Muscle-Nerve-Muscle Grafting for Facial Reanimation in Rats

***James Park*[*], Jeff Hotaling[*], Josh Sappington[*], Grant Turek[*], Steven Charous[*], and Eileen Foecking[†]**

[*]Loyola University Chicago – Stritch School of Medicine, Maywood, IL; [†]Edward Hines Jr. VA Hospital, Maywood, IL

Objective/Hypothesis: Facial paralysis is a devastating condition which leaves patients with myriad aesthetic and functional consequences. Muscle-nerve-Muscle (MNM) neurotization is a reinnervation technique which involves implanting an autogenous nerve graft as a conduit between an innervated "donor" muscle and a denervated "recipient" muscle. Axonal sprouting is subsequently induced whereafter stimulation of the donor muscle results in simultaneous contraction of the recipient muscle. Herein, we investigate use of the MNM reinnervation, alone or in combination with combined electrical stimulation and exogenous testosterone, on the functional recovery following rat facial nerve injury. **Study Design:** Prospective Controlled Animal Study **Methods:** 24 male, Sprague-Dawley rats (200g) were randomly assigned to one of 3 treatment groups of 8 rats each: No graft (Control), MNM Grafting alone (MNM), or MNM Grafting+ electrical stimulation (ES) and testosterone propionate (TP) (MNM+). Each rat underwent microsurgical harvest of the branches of their right facial nerve innervating their whisker pad with subsequent treatments applied as dictated by their treatment group. Functional recovery of whisker movement was then assessed by daily behavioral observations, electromyographic

recordings and video analysis. **Results:** MNM grafting not only improved muscle tone but enhanced vibrissae movement. Animals with the MNM had significantly decrease recovery time for coordinated movement. The application of ES+TP demonstrated further enhancement whereby it significantly improved muscle tone as observed by nose asymmetry as well as recovery time for coordinated movement. At the end of the 16 week study, none of the control animals had displayed coordinated movement while 71% and 85% of the MNM and MNM+ animals respectively did recover coordinated movement. Electromyographic recordings demonstrated electrical conductance across the grafts of both the MNM and MNM+ animals.**Conclusion:** This study is the first to demonstrate that reinnervation following MNM grafting can improve both vibrissae muscle tone and movement following a facial nerve injury while the combinatorial therapy further enhances the recovery of these functional measures. These data have exciting clinical implications for patients with unilateral paralysis as a result of facial or laryngeal nerve injury.

137

Tcfap2c Regulates Oncogenesis and Tumor Growth in Neu-Activated Breast Cancer

***Park JM*[*], Wu T[*], Woodfield GW[*], Cyr AR[*], De Andrade J[*], Zhang W[*], Domann FE[*], Weigel RJ[*]**
[*]University of Iowa, Iowa City, IA

Introduction: TFAP2C and mouse homolog TCFAP2C are transcription factors that play a critical role in mammary gland development and in maintaining the luminal breast cancer phenotype. Interestingly, several human HER2-positive breast cancer cell lines display genetic dependency on TFAP2C. However, the role of TFAP2C in carcinogenesis and progression in Neu-activated breast cancer remains unknown. **Methods:** Mice expressing MMTV-Neu with and without mammary gland conditional knockout (KO) of Tcfap2c were generated. The time until palpable tumor formation was measured in mice harboring the Neu oncogene with and without Tcfap2c. Immunohistochemistry was used to characterize tumors. Paired cell lines from an animal expressing activated Neu with with differential expression of Tcfap2c were established by infecting the cells with Adenovirus-Cre (Ad-Cre) or Adenovirus-Empty (Ad-Empty). Proliferation was assessed by MTT. ChIP-seq and RT-PCR were used to elucidate TCFAP2C targets. **Results**: Mice with homozygous conditional deletion of Tcfap2c demonstrated a significant delay in tumor formation compared to littermate controls that retained Tcfap2c expression (42 vs. 25 weeks, $p<0.004$). Immunohistochemistry of the tumors demonstrated a luminal phenotype; cytokeratin 8 (CK8), a luminal marker, was more strongly expressed compared to cytokeratin 5 (CK5), a basal marker, in tumors from both control (883 vs. 0.1 cells/HPF, $p<0.0005$) and KO animals (845 vs. 0, $p<0.0005$). Cleaved caspase-3 (CC3) showed that apoptosis was increased in tumors from KO compared to control animals (5 vs. 0.3 CC3-positive cells/HPF, $p<0.0008$). Ki-67 showed that proliferation was decreased in KO compared to control (348 vs. 142 cells/HPF, $p<0.009$). MTT demonstrated that loss of Tcfap2c expression resulted in a 30% decrease in proliferation rate ($p<0.006$). Athymic mice that were xenografted with the paired cell lines (Ad-Cre and Ad-Empty) demonstrated that KO of Tcfap2c resulted in a significant delay in tumors reaching a 2 cm threshold (27.8 vs. 19.8 days post-inoculation, $p<0.003$). We identified Erbb3 and Egfr as two TCFAP2C target genes by ChIP-seq. KO of Tcfap2c resulted in a 3-fold and 12-fold reduction in Erbb3 and Egfr expression, respectively ($p<0.05$). **Conclusion:** Tcfap2c appears to positively regulate known oncogenes such as Erbb3 and Egfr. Taken together, these findings indicate that Tcfap2c plays a critical role in oncogenesis and progression of HER2-positive mammary cancer.

138

Personalized Predictions of Macrophage Mediated Breast Cancer Cell Invasive Potential Using Systems Biology

***K-Y Park* and MO Platt**
Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA

Patient variability in breast cancer progression has complicated treatment decisions. Tumor-associated macrophages and cathepsin cysteine proteases they produce promote tumor progression. We previously quantified patient-to-patient variability in monocyte-derived macrophages (MDMs) and osteoclasts cathepsin production and predicted these differences with systems biology analysis of monocyte kinase signaling networks. From those findings, we hypothesize that variability in protease activity of MDMs in tumors may contribute to variability in breast cancer invasiveness, which may be predicted with similar approaches. To test this, primary monocytes from healthy donors were differentiated into MDMs, then co-cultured with MCF-7 breast cancer cells to measure invasion through collagen gels. We also measured intracellular and secreted cathepsins K, L, S and V activity with cathepsin zymography; levels of cathepsin inhibitors cystatin C and B with Western blotting; and kinase phosphorylation of differentiating monocytes with Bioplex. Of the 5 patients, 4 of their MDMs increased MCF-7 cell invasion with a 4-fold difference among the patients. We identified active cathepsin to cystatin C ratio of MDMs, not protease activity alone, to be most predictive of MDM-mediated cancer cell invasion, with 95% predictability, even with the range of variability among these patients. Inhibition of JNK, implicated in cathepsin regulation, resulted in patient-specific responses: cathepsin K was increased in patient 1 but decreased in patient 2, whereas cathepsin V was decreased in patient 1 but increased in patient 2. Our results suggest that patient-specific kinase analysis of monocytes could be useful in clinics to predict cancer invasive potential and may inform personalized chemotherapy.

139

Cardiac and Cutaneous Disease Mutations in DP Misregulate Cx43 by Impairing DP-EB1 Interaction or Localization

***DM Patel*[*] and KJ Green[*,†]**
Departments of Pathology[*] and Dermatology[†], Northwestern University Feinberg School of Medicine

Desmosomes are intercellular junctions that are critical to structural integrity of tissues such as the skin and heart.

Desmoplakin (DP) is an obligate component of desmosomes known to tether intermediate filaments to junctions; however, recent work has demonstrated that DP also regulates microtubule (MT) organization. Through an unbiased screen, we uncovered a novel interaction between DP and EB1, a microtubule plus-end binding protein that has emerged as a major regulator of MT dynamics. We found that DP provides spatial cues for EB1 and MTs to localize to sites of cell-cell contact, where MT plus-ends are stabilized against depolymerization. Our binding analyses indicate that EB1 associates with the DP N-terminus, which contains a hotspot for pathogenic mutations associated with arrhythmogenic right ventricular cardiomyopathy (ARVC). We show that a selection of ARVC mutations compromise EB1 binding and impair membrane localization of Cx43, a gap junction subunit previously reported to use EB1 for MT-based trafficking to the plasma membrane. Further, we found that a mutation in the DP N-terminus associated with skin fragility/woolly hair syndrome caused aberrant DP localization to impair EB1-guided Cx43 membrane localization. Our work thereby identifies a novel mechanism by which MTs interact with intercellular junctions, and highlights the potential importance of the DP-EB1 interaction in the development of cardiac and cutaneous diseases.

140

Tumor Necrosis Factor Alpha and Tissue Factor: Interplay Between Inflammation and Coagulation in Sepsis Associated Coagulopathies

Eric Pedone, **Chris Low, Dan Syed, Jawed Fareed, Debra Hoppensteadt, Mark Cichon, Michael Mosier**
Loyola University, Chicago, IL

Introduction: Sepsis Associated Coagulopathy (SAC) is a serious hemostatic dysfunction which involves simultaneous activation of both coagulation and fibrinolytic pathways. It is associated with high mortality due to end organ failure from microvascular thrombosis. Presently, the only effective management of DIC is to identify and treat its underlying cause, thus making early detection invaluable in improving prognosis. This study seeks to quantify and correlate the levels of two biomarkers, namely Tissue Thromboplastin (Tissue Factor) and Tumor Necrosis Factor Alpha (TNFα), as well as to study the pattern of Thrombin generation in SAC patients, compared to normal human plasma. **Materials and Methods:** Citrated whole blood samples were collected from 49 deidentified SAC patients that had been enrolled as part of a global, double-blind phase 2b study of recombinant Thrombomodulin, as well as 12 healthy volunteers. SAC and NHP samples were analyzed both for TNFa and TF using Zymutest ELISA kits, as well as for Thrombin generation, using a Technothrombin TGA kit. Results were expressed as mean concentration ± Standard Error of the Mean (SEM). Thrombin generation was reported as an area under the curve (AUC) value, as well as peak generation for SAC and NHP. **Results:** A considerable increase was noted in both TNFα and TF in SAC samples, with a 20-fold increase in TF in comparison to NHP (2.91 ± 0.83 pg/mL compared to 0.8665 pg/mL in NHP) and a 45-fold increase in TNFα (15.32 ± 1.462 pg/mL compared to 0.3300 ± 0.1300pg/mL for NHP). Thrombin generation was also remarkable with a 5-fold increase in the AUC value of SAC compared to NHP (573 ± 35.62nM compared to 86.18 ± 27.56nM for NHP). **Discussion:** The results of this study serve to further confirm the interplay that exists between inflammation and coagulation. The sepsis PPP pool showed a dramatic increase in TNFa concentrations, as would be expected in a state of infection/inflammation. However, there was also a remarkable increase in thrombin generation compared to NHP, which, when considered along with the increase in Tissue Factor, demonstrates just how profound of a coagulation response can occur in these patients. The time dependent TGA assay showed not only differing amounts of thrombin produced between SAC and NHP, but also the *pattern* in which it is synthesized. SAC samples showed an abrupt increase in thrombin generation within the first 15 minutes after initiation of coagulation, followed by an equally abrupt decline in synthesis below even the NHP levels. This finding is likely due to the rapid consumption of thrombin exhibited in abnormal hemostatic environment of SAC and helps to elucidate the aberrant regulation of coagulation in these patients.

141

ClinicalTrials.gov as a Data Source for Point-of-Care Trial Recruitment

***PB Pfiffner*[*†], J Oh[‡], TA Miller[*†], KD Mandl[*†]**
Boston Children's Hospital Informatics Program at Harvard-MIT Health Sciences and Technology, Boston, MA; Center for Biomedical Informatics, Harvard Medical School, Boston, MA; Wellesley College, Wellesley, MA

Background: Recruiting patients to clinical trials is an expensive, time consuming, and increasingly difficult process. With high market penetration of electronic medical records (EMR), the physician at the point of care could play a key role in identifying and recruiting patients, aided by intelligent tools comparing trial eligibility criteria to patient EMR data. With the mandate of prospective clinical trial registration, ClinicalTrials.gov (CTG) could become an engine not only of trial registration, but also enrollment. The database could be queried at the point of care and eligibility criteria compared against EMR data and physician and patient input. Eligibility criteria in CTG are provided as free text, however reliable automated eligibility evaluation requires criteria data to be "readable" by a computer. **Objective:** We sought to evaluate the current state of trial registration data in CTG with respect to utility for automated trial matching. **Methods:** We downloaded eligibility criteria and metadata for 437 trials that were open for recruitment in four different clinical areas. The trials were evaluated for currency of their recruitment status and their eligibility criteria were assessed for 4 obstacles to automated text interpretation. Finally, we tried to contact a subset of the trials by phone or email to determine the accuracy of contact details. **Results**: A quarter of the trials were declaring to be still recruiting patients while listing a completion date in the past, indicating out of date records. Additionally, we found each of our 4 barriers to automated text interpretation to be present in 25% to up to 55% of all trials. Lastly, 31% (45 of 146) of the trials in our subset were reachable neither by phone nor by email. **Discussion and Conclusion:** Outdated trial data in CTG is a problem previously identified and further specified by our findings of conflicting recruitment status data as well as incomplete and inaccurate contact details. Trials wishing to promote automated matching must thus ensure to keep registration status up to date and provide accurate contact data to enable referral of potentially eligible patients. Furthermore, our evaluation of obstacles posed to automated eligibility criteria interpretation indicates that such

undertaking is beyond the state of the art for current text analysis software. We argue that enabling trial coordinators to supply key eligibility criteria in a simple yet standardized form to CTG might provide fertile grounds for informatics tools to assist with initial eligibility screening at the point of care.

143

Interleukin-21 Drives Intestinal Inflammation by Bridging the Adaptive and Innate Immune Compartments

CH Poholek, **LE Harrington**
University of Alabama at Birmingham, Birmingham, AL

Objectives/Specific Aims: Th17 CD4 T cells have been cast as pathogenic in the context of many autoimmune diseases, including Inflammatory Bowel Disease (IBD); however, the mechanism for this pathogenicity has yet to be elucidated. Based on the observation that IL-21 expression is increased in biopsies from patients with ulcerative colitis compared to healthy controls and Genome Wide Association Studies have shown an association between the locus containing *il2/il21* and IBD, we posit that IL-21 produced by Th17 cells mediates inflammation in the intestine. **Methods/Study Population:** We use mouse models of both spontaneous and CD4 T cell-dependent colitis to interrogate the role of IL-21 signaling on disease pathogenesis. Analysis is completed using a combination of flow cytometry, ELISA, quantitative-PCR, and histological techniques. **Results/Anticipated Results:** We show that a large number of IL-21-producing CD4 T cells are present in the intestines of mice with colitis and importantly, that IL-21 production is required for full disease progression. We find that although IL-21$^{-/-}$ CD4 T cells are unable to initiate disease, they produce elevated levels of IL-17A and IL-17F and are not selectively converted into Foxp3+ regulatory T cells, indicating that IL-21 is both crucial to the induction of colitis and surprisingly, is not acting via effects on the activation and differentiation of T cells. In direct support of this idea, IL-21R$^{-/-}$ CD4 T cells are capable of inducing disease unlike their IL-21$^{-/-}$ counterparts. In fact, our data demonstrate that IL-21 has an impact on the innate lymphoid cell compartment, which has previously been implicated in IBD pathogenesis. **Discussion/Significance Of Impact:** Taken together, our data show an important and previously unrecognized role for IL-21 that links CD4 T cells to innate lymphoid cells in the induction of chronic intestinal inflammation.

144

Network Measures Predict Locations Where Lesions Especially Disrupt Cognition

***J Power*[1*], D Warren[2], C Lessov-Schlaggar[1], B Schlaggar[1], J Bruss[2], N Denburg[2], D Tranel[2], S Petersen[1]**
[1]Washington University School of Medicine, St. Louis, MO; [2]University of Iowa School of Medicine, Iowa City, IA; [*]MD-PhD Program, Washington University in St. Louis

In this work we use behavioral and imaging data from neurological patients with focal, stable brain lesions to test a set of predictions about how the locations of brain lesions relate to the extent and severity of resultant cognitive and behavioral deficits. These predictions stem not from traditional neuropsychological principles but instead from analyses of the human brain's network organization as revealed by spontaneous low-frequency fluctuations in fMRI BOLD signal in healthy human adults (resting state fMRI). The predictions are as follows. Particular locations in the brain are *articulation points* with multiple closely apposed functional systems (e.g., the visual system, the default mode system, etc). Often, regions within several of the apposed systems, adjacent to these special locations, exhibit correlations across multiple systems. Lesions that involve articulation points and nearby integrative regions are predicted to detrimentally impact integrated processing across multiple systems, and thus are predicted to produce deficits across multiple cognitive and behavioral domains. By contrast, lesions to regions with few proximal systems are predicted to cause more circumscribed cognitive and behavioral deficits. In a review of 30 patients who have damage to either articulation points (6 locations, n=19) or "control" locations associated with single functional systems (2 locations, n=11), we find evidence strikingly consistent with our predictions. Lesions encompassing articulation points uniformly produced severe and wide-ranging deficits to most or all of the 9 cognitive/behavioral domains that were quantified with formal neuropsychological data. In patients with lesions to control locations, deficits were much more limited, rarely involving more than 1 or 2 of the domains, and typically being of mild severity at worst. Dramatic deficits observed at articulation points are not expected under conventional neuropsychological principles but are consistent with predictions derived from the systems-level functional network organization of the brain.

145

Increasing Concentrations of Histone Deacetylase Inhibitor, Suberoylanilide Hydroxamic Acid (SAHA) Decrease Classical Inflammatory Markers in Raw Cells

Samuel Quaynor, **Donald E. Kimbler, John R. Vender, Sangeetha Sukumari Ramesh, and Krishnan M. Dhandapani**
Department of Neurosurgery, Medical College of Georgia, Georgia Regents University, Augusta, GA

Background: Traumatic brain injury (TBI) is a leading cause of death and disability worldwide. Cerebral edema increases intracranial pressure and contributes to a poor patient prognosis after TBI. Unfortunately, surgical treatment options remain limited and medical treatment options are lacking. Recent work by our laboratory suggests macrophage-derived inflammatory mediators promote the development of post-traumatic brain edema. **Hypothesis**: Suberoylanilide hydroxamic acid (SAHA), a FDA-approved pan-histone deacetylase inhibitor (HDACi) will attenuate immune activation and reduce cerebral edema after TBI. Methods: Studies were conducted in an established pre-clinical mouse model of controlled cortical impact, which mimics a moderate TBI in humans. SAHA (25-100 mg/kg) was administered via an intraperitoneal route at the time of injury. Edema was assessed using the wet-dry method. Further mechanistic work was performed in the RAW264.7 macrophage cell line. **Results:** Administration of 75 or 100 mg/kg SAHA significantly increased cerebral edema at 24h post-TBI, as compared to placebo-

treated mice, whereas lower doses of SAHA were ineffective. The reduction in edema was paralleled by a concomitant increase in acetylation status within the peri-contusional cortex, consistent with the proposed function of SAHA as an HDACi. At different concentrations, SAHA either increased or decreased the expression of classical inflammatory markers, such as IL1β, IL6 and IL10, in activated macrophage cells *in vitro*. **Conclusion:** HDACi may represent a novel class of drugs to reduce cerebral edema following TBI, in part, via the modulation of immune activation.

146

Activation of Alternative Receptor Tyrosine Kinases in PDGF-driven Glioblastoma Mediates Resistance to PDGFR Inhibition

***Cong Ran*†*, **Zhonghua Zhang*†, Mark A. Israel*†**

[*]Departments of Pediatrics and Genetics, Norris Cotton Cancer Center[1], Geisel School of Medicine at Dartmouth[2], Hanover, NH

Receptor tyrosine kinase (RTK) pathways including the pathways mediated by PDGFR and EGFR have been implicated in the pathogenesis of glioblastoma multiforme (GBM). The use of RTK inhibitors has thus been evaluated in the treatment of GBM; however, such therapy has not improved patient survival because therapeutic resistance becomes rapidly evident. Our laboratory has described a mouse model of glioma that mimics the features of human proneural GBM, a subtype of GBM that is closely associated with PDGFR activation. Exposing these mice to chronic PDGFR inhibition, we identified PDGFR-inhibition resistant tumors that required the activation of insulin receptor (IR)/insulin growth factor receptor (IGF1R) for growth and survival. In spheroid cultures (TSC) derived from resistant tumors, we found that IR/IGF1R-promoted cellular growth required activation of PI3K/AKT and MAPK. PI3K/AKT pathway mediated survival, which can be active by both IR/IGF1R and PDGFR is important in TSCs, suggesting a role for IR/IGF1R in resistance developed in chronic PDGFR-inhibition. Co-targeting IR/IGF1R and PDGFR reduced the emergence of TSCs resistant to PDGF/PDGFR inhibition. Evaluating public databases describing the molecular changes in GBMs, we found evidence to support that IR/IGF1R plays important role in PDGFR-driven human GBM, which may be selected as resistance mechanism in the corresponding treatment condition, such as PDGFR inhibition. These findings indicate the importance of IR/IGF1R activation in mediating resistance to PDGFR-inhibition in a mouse model of GBM and suggest a role for inhibition of IR/IGF1R in the treatment of glioma driven by PDGF/PDGFR activation.

147

Monocytic Cells Mediate Granulocyte-Colony Stimulating Factor-Induced Mobilization of Hematopoietic Stem and Progenitor Cells

***M. Rao*[1,2], T. Supakorndej[1,2], M.J. Christopher[1], J. Woloszynek[1], F. Liu[1], and D.C. Link[1]**

[1]Department of Internal Medicine, Division of Oncology, Washington University in St. Louis; [2]MD-PhD Program, Washington University in St. Louis

In the normal, healthy adult, hematopoiesis occurs primarily in the bone marrow. As such, at steady state, a majority of hematopoietic stem and progenitor cells (HSPCs) are tightly held within specialized areas of the bone marrow collectively referred to as the 'hematopoietic stem cell niche.' In response to various forms of stress, such as infection or hemorrhage, or following administration of a variety of pharmacologic agents, niche function is altered and HSPCs are released into the peripheral circulation through a process known as mobilization. Granulocyte colony stimulating factor (G-CSF), a hematopoietic growth factor, is the most widely-used mobilizing agent clinically. However, the mechanisms by which G-CSF elicits HSPC mobilization are not well-understood. The experiments described are directed towards understanding of regulation of HSPC trafficking between the bone marrow and periphery. We first focused on identifying the hematopoietic lineage directly targeted by G-CSF in the initiation of HSPC mobilization. Using a mouse model in which the G-CSF receptor is selectively expressed on monocytic cells, we demonstrate that G-CSF signaling in monocytic cells is sufficient for a normal mobilization response. Subsequent studies were designed to identify which of the three major cell types within the monocytic lineage – bone marrow macrophages, osteoclasts, and myeloid dendritic cells – are critical for retention of HSPCs within the bone marrow and for the mobilization response to G-CSF. We demonstrate that loss of myeloid dendritic cells does not abrogate the mobilization response to G-CSF and that pharmacologic ablation of osteoclasts does not lead to HSPC release into the periphery. Lastly, we evaluated the importance of selected factors produced by monocytes in the mobilization response to G-CSF. We demonstrate that hematopoietic loss of osteopontin does not alter the mobilization response to G-CSF. Further work will be required to evaluate the role of bone marrow macrophages and myeloid dendritic cells in HSPC retention as well as the requirement for osteoclasts in G-CSF-induced mobilization. Future studies will be directed at identifying the factors used by monocytic cells to communicate with the stromal components of the HSPC niche during G-CSF-induced mobilization.

148

Pancreatic Cancer Cells Developing Resistance to BET Bromodomain Inhibitor JQ1 Upregulate ZEB1 and Demonstrate Epithelial-Mesenchymal Transition

***SS Raza*, LM Knab, K Kumar, CR Chow, K Ebine, HG Munshi**

Department of Medicine and the Robert H. Lurie Cancer Center, Feinberg School of Medicine of Northwestern University, Chicago, IL

There is increasing interest in developing inhibitors targeting epigenetic changes, including targeting BET proteins that bind acetylated histones to regulate gene transcription. We have shown the BET-inhibitor JQ1 decreases growth of pancreatic cancer cells and represses c-MYC and FOSL1 mRNA expression. Since cells can develop resistance to chemotherapy and targeted therapies, we treated pancreatic cancer cells with increasing concentration of JQ1 to develop JQ1-resistant cells. We were able to successfully create one cell line that continued to grow at 10x the IC50 for JQ1 (CD18-JQ1^{r}). We also generated CD18 cells resistant to 5-fluorouracil (CD18-CR). Although CD18-JQ1^{r} cells had similar c-MYC levels as parental CD18 cells (CD18-P), CD18-JQ1^{r} cells showed upregulation of FOSL1. These cells also showed evidence of epithelial-mesenchymal transition, with repression

of E-cadherin, repression of miR200 family of microRNA and upregulation of ZEB1. Transfection of CD18-JQ1^r cells with ZEB1 siRNA reversed the phenotypic changes, and sensitized CD18-JQ1^r cells to JQ1. In contrast to CD18-CR cells, CD18-JQ1^r cells demonstrated lower levels of a2-integrin expression, reduced FAK phosphorylation and reduced adhesion and spreading onto collagen-coated surfaces. Activation of integrins in CD18-JQ1^r cells enhanced adhesion and spreading onto collagen-coated surfaces, similar in extent to CD18-CR cells. Overall, these results suggest that cells can develop resistance to BET inhibitors and the resistant cells have defects in both cell-cell and cell-matrix adhesion.

149

Targeting β-arrestin2 Enhances Survival in a Murine Model of Chronic Myeloid Leukemia

***LM Rein*[*], M Chen[*], BS Theriot[*], JW Wisler[*], LM Wingler[*], RT Premont[*], W Chen[*], JLK Walker[*], and RJ Lefkowitz[*†]**
[*]Duke University, Durham, NC; [†]Howard Hughes Medical Institute, Durham, NC

Differentiated BCR-ABL positive chronic myeloid leukemia (CML) cells can be eliminated with tyrosine kinase inhibitors (TKIs). Despite targeted therapy, CML stem cells are not completely eradicated and renew and proliferate independent of BCR-ABL. In addition, mutations can confer resistance to TKIs and are associated with poor prognosis. β-arrestins are multifunctional adaptor proteins that have been implicated in several signaling pathways associated with CML. We hypothesize that β-arrestin2 (βarr2) is necessary for development and propagation of CML and could be a therapeutic target in chronic phase (CP) CML and TKI-resistant CML harboring a T315I mutation. **Methods:** We modeled CP BCR-ABL(+) CML with a murine retroviral transduction system. KLS cells (Lin^-,$Sca\text{-}1^+$,$ckit^+$) were harvested from marrow of donor CD45.2 conditional C57BL6/J $\beta arr2^{F/F}$-$CreERT2^{+/-}$ (Cre (+)) and control C57BL6/J $\beta arr2^{F/F}$-$CreERT2^{-/-}$ (Cre (-)) mice. Cells were transduced with MSCV-BCR-ABL-IRES-GFP and were injected into sublethally irradiated wild type (WT) recipient mice (CD45.1). Tamoxifen was initiated on day 3 after transplant to induce βarr2 knockout and mice were monitored for leukemia. The primary outcome was survival in recipient mice receiving Cre (+) versus Cre (-) cells. Secondary outcomes included white blood cell (WBC) count, number of donor cells present in recipient mice, and spleen size. Hematopoietic cell growth and differentiation was assessed with methylcellulose assays using KLS cells from WT and $\beta arr2^{-/-}$ mice. Cells were infected with mutant MSCV-BCR-ABL^{T315I}-IRES-GFP virus versus vector and colonies formed were counted after 12 days. **Results:** Ten of 11 (90.0%) Cre (-) mice developed CML as evidenced by splenomegaly, increase in number of detectable donor cells, and leukocytosis. Median survival was 15 days. Six of 8 (75%) Cre (+) mice developed CML with median survival of 27 days (HR 3.2, 95% CI;1.525-12.2, p=0.013). At day 11, flow for donor cells was 38 ± 2.05% in Cre (-) versus 16.5± 3.9% in Cre (+) mice ($p<0.0001$). In methylcellulose assays, 48.8% fewer colonies formed from cells infected with T315I mutant in $\beta arr2^{-/-}$ versus WT cells indicating that βarr2 is necessary for colony formation in resistant CML as we have previously seen in CP CML. **Conclusions:** These data demonstrate that targeting βarr2 prolongs the CP CML disease course and suggest that βarr2 affects CML development in resistant disease. βarr2 may therefore represent a therapeutic target for CML independent of tyrosine kinase inhibition.

150

Mutation of a Zinc Finger Polyadenosine RNA Binding Protein, ZC3H14, Causes Autosomal Recessive Intellectual Disability

***J Rha,* SW Leung, GJ Bassell, AH Corbett**
Emory University School of Medicine, Atlanta, GA

Intellectual disability affects between 1-3% of people across the world. Patients with intellectual disability suffer from significantly subaverage intellectual function (IQ ≤70), which impinges on quality of life. In a collaborative effort, we have recently identified the first gene encoding a polyadenosine RNA binding protein, ZC3H14 (Zinc finger CysCysCysHis domain-containing protein 14), which is mutated in inherited nonsyndromic autosomal recessive intellectual disability. This finding uncovers the molecular basis for disease in these patients and provides strong evidence that ZC3H14 is essential for proper brain function. ZC3H14 is an evolutionarily conserved member of a novel class of tandem zinc finger (CCCH) polyadenosine RNA binding proteins. Studies of ZC3H14 orthologs in budding yeast and *Drosophila* provide insight into the role of this protein in post-transcriptional regulation of gene expression, specifically in the proper control of 3′-end polyadenylation of mRNA. Despite studies in yeast and *Drosophila*, functional characterization of ZC3H14 in vertebrates is crucial for understanding the role of this protein in brain function and the molecular mechanism underlying intellectual disability in these patients. Therefore, we have developed a conditional *ZC3H14* knockout mouse utilizing the Cre/loxP system to extend our studies to vertebrate ZC3H14 and address our hypothesis that ZC3H14 is required for proper expression of target mRNAs that are critical for neuronal function. We utilized the EIIa-Cre transgenic line, in which Cre-recombinase is expressed ubiquitously, to knockout ZC3H14 expression. EIIa-Cre +, ZC3H14 -/- mice are viable, suggesting that *ZC3H14* is not essential. These *ZC3H14* knockout mice provide us with an optimal model to study the requirement for ZC3H14 in higher order brain function. We are currently performing preliminary analyses to define molecular and functional effects of the loss of ZC3H14 by examining aspects of neuronal morphology, overall brain architecture, and cognitive behavior. Additionally, we will extend our observations from yeast and *Drosophila* and specifically investigate the role of ZC3H14 in 3′-end polyadenylation of mRNA for the first time in the mouse model organism. By understanding how ZC3H14 regulates target mRNAs—and thus their expression—in mice, we can begin to define the role of ZC3H14 in for normal brain function. Our long-term goal is to understand how dysregulation of post-transcriptional control of mRNA in neurons leads to neuronal dysfunction and consequently impaired brain function.

Poster Abstracts

151

Imatinib Attenuates Endothelial Damage in LPS Induced Lung Injury *in vitro* and *in vivo* Rationale

A Rizzo, **E Letsiou, S Sammani, P Naureckas, J Jacobson, J Garcia, S Dudek**
University of Illinois College of Medicine at Chicago, Chicago, IL

Acute lung injury (ALI) is characterized by inflammation-induced disruption of the endothelial cell (EC) barrier lining the pulmonary vasculature, which causes leakage of fluid, protein, and inflammatory cells into the airspaces. The mechanisms underlying ALI are critically dependent on EC cytoskeletal regulation of vascular permeability. Recently published work indicates that the tyrosine kinase Abl regulates the cytoskeleton to maintain vascular integrity. Conversely, the closely related kinase abl related gene (arg) contributes to endothelial barrier disruption. The pharmaceutical agent imatinib inhibits both Abl and Arg and is used to treat patients with chronic myelogenous leukemia and other malignancies. However, numerous case reports have established that imatinib can cause significant vascular leak and edema. Given these effects on permeability and current clinical usage, we sought to determine the effects of imatinib in LPS-induced ALI *in vitro* and *in vivo*. **Methods:** Transendothelial electrical resistance (TER) was measured in LPS-challenged human pulmonary artery ECs treated with imatinib or vehicle. Complementary western blots (WB) for EC junctional proteins (VE-Cadherin) and inflammatory markers (VCAM) were conducted. Abl or Arg expression was selectively decreased via siRNA, and the LPS responses were assessed. C57BL6 mice received intraperitoneal imatinib (75 mg/kg) or saline either immediately prior to, or 4 hours after, intratracheal LPS (1 mg/kg). After 18 hours, bronchoalveolar lavage (BAL) and lungs were harvested. Lung injury was assessed by BAL cell counts, protein content and cytokine levels, as well as Evans blue dye extravasation and histologic lung injury scoring. **Results**: Imatinib inhibits the LPS induced decrease in transendothelial electrical resistance (Figure 1). Imatinib dose-dependently decreased production of inflammatory cytokines (IL6 and IL8) and expression of VCAM in LPS-challenged ECs. Additionally, imatinib restored VE-Cad expression in LPS-challenged ECs. Silencing Abl or Arg attenuated LPS-induced VCAM expression in ECs. Additionally, imatinib treatment, both pre- and post- LPS challenge, significantly decreased LPS-induced vascular leak and inflammatory response in mice. LPS challenged mice that received imatinib pre-treatment had a decrease in total cell counts of 59% (p=0.025) and a decrease in BAL protein of 53% (p=0.0015). **Conclusions:** Imatinib dose-dependently inhibits LPS-induced inflammation and vascular leak *in vitro* and *in vivo*. These effects may be mediated in part via the tyrosine kinases Abl and Arg, which play a critical role in the regulation of the EC cytoskeleton. Thus, imatinib may be a potential therapeutic option for patients with ALI.

152

Role of Glutamate Transporters in Redox Homeostasis

SM Robert, **TA Ogunrinu Barbarinde, KT Holt, H Sontheimer**
University of Alabama at Birmingham, Birmingham, AL

Glioblastoma multiforme is the most prevalent malignant brain tumor. Past research has identified an important role for the cystine/glutamate exchanger, system x_c^- (SXC), in the growth of these tumors, providing cellular uptake of cystine towards synthesis of the endogenous antioxidant glutathione (GSH). In glioma cell lines, SXC was shown to be the exclusive pathway for cystine uptake and GSH synthesis. To better replicate human disease, we examined patient-derived glioma xenolines and found only ~50% of tumors have high SXC expression on Western blot. Interestingly, those with low/absent SXC expression instead expressed the glutamate transporters EAAT1 and/or EAAT3, previously found absent/nonfunctional in glioma cell lines. Surprisingly, resting GSH levels in tumors with high- versus low-SXC levels showed no significant difference. We resolved this conundrum by studying ^{35}S-cystine uptake, which revealed Na^+-dependent, TBOA-sensitive cystine uptake in these cells, suggesting that EAAT1 and/or EAAT3 act as alternate pathways for cystine uptake and GSH synthesis. Since GSH protects cells from reactive oxygen species (ROS) damage, we questioned whether gliomas that import cystine via different pathways exhibit differential sensitivity to radiation therapy, which relies upon the production of the hydroxyl free radicals that GSH effectively neutralizes. We found at clinically relevant doses (1G), high-SXC expressing xenolines exhibited a significantly greater resistance than low-SXC expressing tumors. SXC-inhibition using sulfasalazine (SAS) decreased resistance in high-SXC tumors, but was ineffective in low-SXC tumors. These findings suggest that low-SXC tumors satisfy baseline GSH requirements via EAAT1/3-mediated cystine uptake; however, they cannot increase cystine uptake and GSH in response to oxidative stress. Indeed, SXC is regulated via hypoxia-inducing factor, which does not regulate EAATs. Defining subpopulations of gliomas with differential radiation sensitivity has important implications for a personalized medicine approach to patient treatment. These findings suggest that only patients expressing SXC would benefit from receiving SAS to enhance radiation therapy.

153

C1013G/CXCR4, A Novel Activating Mutation in Lymphoplasmacytic Lymphoma

A.M. Roccaro, **A. Sacco, C. Jimenez, P. Maiso, M. Moschetta, Y. Mishima, J.San Miguel, R. Garcia-Sanz, I.M. Ghobrial**
Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA

The C-X-C chemokine receptor type 4 (CXCR4) plays a crucial role in modulating the biology of B-cell lymphoproliferative disorders. We sought to define the mutational status of CXCR4 in B-cell malignancies. 418 patients with different B-lymphoproliferative disorders were studied. The *C1013G/CXCR4* variant was detected in 28% of patients with Waldenstrom Macroglobulinemia (WM; 37/131); 20% of IgM MGUS (8/40); 7% of splenic marginal zone lymphoma (1/14); 1.3% of diffuse large cell lymphomas (1/75); being absent in B-CLL, hairy cell leukemia, multiple myeloma,

Poster Abstracts

IgG/IgA MGUS, lymphoplasmacytic lymphoma without WM criteria and in healthy individuals. The functional relevance of the C1013G/CXCR4 variant was next examined *in vivo*, demonstrating its activating role in WM cells, as documented by significant tumor proliferation and dissemination to extramedullary organs (liver, bone marrow, lymph nodes, kidney, lung) leading to disease progression and decreased survival. Immunohystochemistry showed the presence of CXCR4+ and CD20+ cells in all the tissues examined; and quantification of CXCR4 and CD20 positivity was higher in *C1013G/CXCR4*-cells-, compared to parental-WM cell-injected mice ($P<0.05$). *C1013G/CXCR4*-cells were further characterized *in vitro*, showing increased adhesion and cell proliferation in the presence of primary WM BM-MSCs. These findings were also confirmed using CXCR4-overexpressing cells. In contrast CXCR4-knockdown cells presented the opposite behavior, where reduced adhesion and proliferation in presence of primary WM BM-MSCs were observed. By performing GSEA we demonstrated that genes related to invasiveness, cell proliferation, anti-apoptosis, and oncogenesis were enriched in *C1013G/CXCR4*-cells compared to the parental-WM cells. These findings let us hypothesize that *C1013G/CXCR4* may act as an activating mutation in WM cells. Indeed, in a different mouse model, CXCR4 over-expressing cells were injected into mice, showing similar phenotype to the one observed upon *C1013G/CXCR4*-WM cell-injected-mice. Finally, the novel antibody anti-CXCR4 led to significant tumor reduction and abrogation of extramedullary dissemination *in vivo*, in a C1013G/CXCR4 WM model. These findings demonstrate that C1013G/CXCR4 is an activating mutation in WM, and support the role of this protein as a critical regulator of WM molecular pathogenesis and as an important therapeutic target.

154

In vivo Targeting of Stromal-Derived Factor-1 as a Strategy to Prevent Myeloma Cell Dissemination to Distant Bone Marrow Niches

A.M. Roccaro, **A.Sacco, M. Moschetta, Y. Mishima, P. Maiso, A. Krushinski, G. Rossi, I.M. Ghobrial**
Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA

Multiple myeloma (MM) patients present with multiple lytic lesions at diagnosis, indicating the presence of continuous dissemination of MM cells from the primary site of tumor development to multiple distant bone marrow (BM) niches. We hypothesized that stromal-derived factor-1 (SDF-1) may represent a target for preventing transition from MGUS (micrometastatic stage) to active-MM (macrometastatic stage); thus resulting in inhibition of MM progression. We evaluated SDF-1 expression in the BM of patients with MGUS, MM, compared to healthy individuals; and tested NOX-A12, a high affinity L-oligonucleotide (Spiegelmer) anti-SDF-1 in MM, using *in vivo* model of MM cell homing and dissemination. Patients with active-MM present with higher bone marrow (BM) SDF-1 expression vs MGUS patients and healthy individuals. Similarly, BM presenting with metastasis from epithelial primary tumors had higher SDF-1 levels compared to healthy subjects, thus suggesting the importance of SDF-1 in favoring tumor cell metastasis to BM niches. SDF-1 co-localized at BM level with MM tumor cells *in vivo*. Similarly, a localization of fluorescently labeled NOX-A12 within MM cell-enriched BM niches was documented using *in vivo* confocal microscopy. Pre-treatment of mice with NOX-A12 resulted in less receptive BM milieu, as demonstrated at mRNA level with down-regulation of genes related to cell growth, cyokine production, angiogenesis and integrin-mediated cell adhesion. This was further corroborated by the demonstration of a chemopreventive effect in mice pre-treated with NOX-A12 compared to control mice: a reduced *in vivo* tumor growth was documented in NOX-A12 pre-treated mice. Moreover, NOX-A12 pre-treated mice presented with reduced MM cell dissemination from bone to bone, as demonstrated using an *in vivo* model of MM cell dissemination. In addition, NOX-A12 used in combination with bortezomib, showed a significant inhibition of MM cell homing, as shown by *in vivo* confocal microscopy. These findings indicate that SDF-1 represents a valid target for inhibiting MM cell dissemination to distant BM niches, thus providing the evidence for using the SDF1 inhibiting spiegelmer NOX-A12 to target MM cells at the stage of micrometastasis (MGUS), thus preventing development of macrometastatic MM.

155

CSF Cytokine Profile of NOMID Patients in Clinical Remission on IL-1 Blocking Treatment, Anakinra

JJ Rodriguez-Smith, **H. Kim, D. Chapelle, G. Montealegre, N. Plass, W. Tsai, Y. Huang, M. Gadina & R. Goldbach-Mansky**
National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH, Bethesda, MD

Background Neonatal Onset Multisystem Inflammatory Disease (NOMID) is an autoinflammatory disease caused by autosomal dominant mutations in NLRP3 that leads to constitutive NLRP3 inflammasome activation and IL-1β release. Patients present with neutrophilic urticaria, bony overgrowth, chronic aseptic meningitis, and papilledema and if untreated develop developmental delay, progressive vision and hearing loss. IL-1β inhibitors resolve the inflammatory disease manifestations, normalize acute phase reactants, decrease cytokine levels, and improve CSF WBC and protein levels. We examined whether NOMID patients in clinical remission have normalized CSF and blood cytokine levels. **Methods** Baseline and post-treatment CSF and plasma/serum(P/S) cytokine levels were assessed via multiplex assay in patients (n=21) and controls (n=7). Post-treatment samples on IL-1 receptor antagonist, Anakinra were available for 19/21 patients, 13 patients fulfilled clinical remission criteria (CRP≤0.5mg/dl, CSF WBC≤5cells/μl and protein≤40mg/dl). **Results** At baseline, CSF and P/S cytokines, IL-6, IL-12(p70), and IL-18 and only CSF IP-10 were significantly elevated compared to controls (n=7). Post treatment, cytokine levels dropped but never normalized even in patients in clinical remission (n=13); CSF and P/S IFNγ and TNFα increased on therapy. CSF/blood cytokine ratios were 6.5 and 4.0 for IL-6 and IP-10 respectively. Post treatment ratios dropped to 0.5 and 1.5 respectively but never normalized. In the CSF IL-6 was 40x higher than control levels and dropped to 2 times higher than normal values. **Conclusions** 1) NOMID patients in clinical remission continue to have evidence of residual cytokine elevation, but long term consequences are currently unknown. 2) CNS cytokine levels do not always reflect P/S levels and suggest local CNS cytokine production and differential response to treatment.

Poster Abstracts

JJRS research was made possible through NIH Medical Research Scholars Program, a public-private partnership supported jointly by the NIH and contributions to the Foundation for the NIH from Pfizer Inc, The Doris Duke Charitable Foundation, The Alexandria Real Estate Equities, Inc. and Mr. and Mrs. Joel S. Marcus, and the Howard Hughes Medical Institute, as well as other private donors.

156

Redox Effects on ERK Promote an M2 Phenotype in Pulmonary Fibrosis via Activation of PP2A

B.H. Rosen, **C. He, A.B. Carter**
University of Iowa Carver College of Medicine, College of Public Health, and Iowa City VA Healthcare System

Rationale: The generation of reactive oxygen species (ROS), such as H_2O_2, by alveolar macrophages has a key role in the pathogenesis of pulmonary fibrosis. Macrophages are characterized by two basic phenotypes: classically (M1) or alternatively (M2) activated macrophages. Overexpression of Cu,Zn-SOD has a role in mediating alternative activation of macrophages *in vitro* and accelerating the development of pulmonary fibrosis *in vivo*. In the presence of Cu,Zn-SOD, macrophages generate increased levels of H_2O_2, which is known to inactivate extracellular signal-regulated kinase (ERK). H_2O_2 activates protein phosphatase 2A (PP2A), a phosphatase that inactivates ERK. ERK activation is critical for inflammatory gene expression. We hypothesized that ERK activity is decreased in alveolar macrophages exposed to chrysotile and inhibition is augmented by overexpression of Cu,Zn-SOD, thus polarizing macrophages to an M2 phenotype and mediating the development of fibrosis. **Methods:** Macrophages were exposed to chrysotile asbestos following overexpression of wild-type Cu,Zn-SOD, constitutively active MEK1 (the upstream kinase of ERK), dominant negative ERK, wild type PP2A, dominant negative PP2A, or empty vector. ERK activity and M1 and M2 gene expression were determined. **Results:** Exposure to chrysotile asbestos decreased ERK activation and overexpression of Cu,Zn-SOD significantly reduced ERK activation. TNF-α promoter activity was significantly greater in macrophages expressing a constitutively active MEK1, whereas promoter activity in cells expressing a dominant negative ERK vector was below control levels. Cu,Zn-SOD increased PP2A activity, and inhibition of PP2A or expression of a dominant negative PP2A increased ERK activity and TNF-α promoter activity. Production of M1 genes, specifically TNF-α, was significantly reduced in cells overexpressing Cu,Zn-SOD despite expression of constitutively active MEK1. In the presence of an inhibitor of PP2A there was decreased expression of M2 genes, CCL-18 and mannose receptor. **Conclusions:** These observations suggest that chrysotile-induced H_2O_2 inhibits ERK activity in alveolar macrophages by activation of PP2A, which limits M1 gene expression. This provides a novel mechanism by which Cu,Zn-SOD-induced H_2O_2 polarizes macrophages to an M2 phenotype and induces the development of a fibrotic phenotype.

157

Dominant Negative *DNMT3A* R882H Mutations in Acute Myeloid Leukemia

D.A. Russler-Germain*†*, **D.H. Spencer***, **M.A. Young***, **T.L. Lamprecht***, **T.J. Ley***
Washington Univ. School of Medicine, St. Louis, MO; †MD-PhD Program, Washington University in St. Louis, St. Louis, MO

Mutations in *DNMT3A* (*de novo* DNA methyltransferase 3A) are found in >30% of normal karyotype AML cases and correlate with poor clinical outcomes. Most *DNMT3A* mutations occur at position R882 within the catalytic domain (most commonly R882H) and are virtually always heterozygous. This over-representation suggests that mutations at R882 may result in gain-of-function or dominant-negative activity that contributes to leukemogenesis. We purified full-length, human wild-type (WT) and R882H DNMT3A using a mammalian tissue culture system to produce recombinant proteins for biochemical modeling of the *de novo* methylation potential of a *DNMT3A*-mutant AML cell. R882H DNMT3A exhibited 21.7% (+/- SD of 4.4%) of the activity of WT DNMT3A in an *in vitro* methylation assay. To simulate an AML cell with a heterozygous R882H mutation, we co-transfected HEK293T cells with equal amounts of poly-His-tagged WT and R882H *DNMT3A* expression vectors and purified both WT and R882H DNMT3A proteins together. The co-purified proteins exhibited a striking 88% reduction in methyltransferase activity, which is similar to the activity of R882H DNMT3A alone and therefore demonstrates a dominant negative effect of this mutation.WT DNMT3A catalytic activity is highly dependent on its ability to form tetramers. We postulated that the dominant negative effect of R882H may involve disruption of WT DNMT3A oligomerization. Using size exclusion chromatography, we identified a tetramerization defect of R882H DNMT3A relative to WT DNMT3A. Notably, co-purified WT and R882H DNMT3A complexes exhibited a pattern of oligomerization identical to R882H DNMT3A alone. Thus, the R882H mutation in DNMT3A not only severely reduces its own *de novo* methyltransferase activity, it also disrupts the ability of WT DNMT3A to form functional tetramers. These two effects severely reduce total DNMT3A activity in AML cells, and may explain why this mutation is virtually always heterozygous in AML samples, since homozygosity would not further reduce DNMT3A activity.

158

Regulation of Mammalian Metabolism by Tristetraprolin

Konrad Sawicki, **M Bayeva, H Ardehali**
Chicago, IL

Heart disease and stroke are the primary causes of death in adults with type-2 diabetes thus strict metabolic control is essential in these patients. Here, we show that tristetraprolin (TTP), a protein involved in regulation of inflammation and iron homeostasis via mRNA degradation, may alleviate diabetic phenotype through modulation of key enzymes involved in gluconeogenesis and fatty acid (FA) metabolism in insulin-sensitive tissues. TTP protein content was significantly reduced in livers of diabetic mice fed high-fat diet compared to control, suggesting that mRNA targets of TTP may be stabilized in diabetes. We then

assessed the effects of TTP knockdown using siRNA in HepG2 liver cells on the expression of enzymes in four major metabolic pathways: glycolysis, gluconeogenesis, FA oxidation and synthesis. Consistent with reduced TTP, we found increased expression of a key gluconeogenic regulator - pyruvate dehydrogenase kinase 4 (PDK4). In silico analysis of the 3' untranslated region (UTR) of PDK4 revealed five putative TTP binding sites. Importantly, elevated PDK4 levels were previously reported in diabetic patients, and are thought to exacerbate the disease by increasing hepatic glucose output. Assessment of FA metabolism revealed increased levels of PPARα and two of its targets, carnitine palmitoyltransferase I (CPT1) and fatty acid translocase (FAT). Consistent with increased FA flux, triglyceride levels were elevated with TTP siRNA in hepatocytes. In silico analysis revealed multiple well-conserved putative TTP-binding sites in the 3'UTR of PPARα, consistent with regulation by TTP. We also observed significant upregulation of PPARα and its targets in HL-1 cardiac cell line, indicating an overlapping function for TTP in insulin-sensitive tissues. Notably, cardiac-specific overexpression of PPARα was previously shown to cause a phenotype resembling diabetic cardiomyopathy, further suggesting that reduction of TTP in diabetes may exacerbate this disease. Our studies show that TTP is downregulated in diabetic mouse livers, and knockdown of this protein in hepatic and cardiac cells increases the levels of key metabolic enzymes, PDK4 and PPARα, both of which are implicated in diabetes and heart disease.

159

HMGN2 Competes with Histone H1 to Facilitate Prolactin-Induced Transcription

***SM Schauwecker*[*], CV Clevenger[†]**

[*]Northwestern University, Chicago, IL; [†]VCU, Richmond, VA

The peptide hormone prolactin (PRL) promotes normal breast tissue growth and maturation, but PRL also contributes to breast cancer development. PRL binds to the transmembrane PRL receptor (PRLr). The PRLr signals from the cell surface to the nucleus both by activating canonical signaling pathways and by directly translocating to the nucleus. In the nucleus, the PRLr promotes gene expression, but the mechanism by which it does so has not been fully elucidated. We have previously shown that nuclear PRLr binds to the chromatin-modifying protein high-mobility group N2 (HMGN2), recruiting HMGN2 to the promoter of a PRL-responsive gene. At the promoter, HMGN2 stimulates transcription, but the mechanism by which HMGN2 does so is unknown. The objective of this study is to determine how PRLr-mediated recruitment of HMGN2 stimulates the transcription of PRL-responsive genes. This knowledge will improve our understanding of how PRL exposure contributes to breast cancer pathogenesis and will also identify targets for novel breast cancer therapies. HMGN2 binds to nucleosomes and induces chromatin decompaction. In particular, HMGN2 can compete with the linker histone H1 for binding to chromatin, antagonizing the chromatin-condensing activity of H1. We hypothesize that HMGN2 causes chromatin decompaction at promoters of PRL-responsive genes, allowing the transcriptional machinery to access the promoter DNA and initiate transcription. In these studies, the promoter of the breast cancer-relevant, PRL-responsive gene *CISH* (cytokine-inducible SH2-containing protein) was examined by ChIP for factors regulating chromatin compaction. Prior to PRL stimulation, the *CISH* promoter was found to exhibit low histone H3 density near the binding site of the transcription factor Stat5a. Stat5a is a critical component of PRLr signaling. PRL stimulation results in decreased H3 density across the *CISH* promoter. PRL stimulation also results in loss of H1 at the CISH promoter. Following HMGN2 knockdown, the loss of H1 is attenuated. Therefore, HMGN2 may compete with H1 for binding to the *CISH* promoter. Consistent with transcriptional activation, PRL stimulation also results in increased RNA Polymerase II (Pol II) bound at the CISH promoter; knockdown of HMGN2 results in less bound Pol II. These data suggest that HMGN2 facilitates PRL-induced transcription by competing with H1 for binding to the promoter DNA, thus promoting chromatin decompaction to allow the transcriptional machinery to better access the promoter DNA. This work is supported by the NIH (F30 CA171858) and a Malkin Scholar Award.

160

The Effect of Ankle Bracing on Proprioception in Functional Ankle Instability: A 3-Dimensional Evaluation of Ankle-Foot Complex Kinematics in Elite Athletes

***Brian Schneiderman,* Michael Chiodo, Marc Angerame, Michael Pinzur**

Department Orthopedics and Rehabilitation, Loyola University of Chicago Health System

Ankle injuries are common in sports and recreational activities. Chronic ankle impairment appears to be a frequent and challenging result of ankle injury. Reports estimate 40-75% of athletes who sustain a lateral ankle sprain will experience residual symptoms. Functional ankle instability (FAI) is one subgroup of these chronic symptoms, characterized by proprioceptive, muscular strength, and neuromuscular control deficits. In the ankle-foot complex, proprioception facilitates the harmonious coordination of resistant and compensatory forces to stabilize the ankle during intricate movements. If proprioception is compromised in the ankle, reinjury becomes more likely. In order to treat for FAI, athletes often tape or brace an affected ankle. Despite their wide use in sports, the effects and value of these orthoses remain controversial. The mechanical benefits of taping have been shown to subside in as little as 20 minutes of exercise, while bracing has proved ineffectual in improving ankle joint strength or stability in some studies. Yet, both taping and bracing have been effective in improving foot position awareness by enhancing proprioception in subjects. However at this time, few studies have been conducted examining the proprioceptive benefits of orthoses on the ankle with the foot free from plantar contact with a surface. The present study aims to examine the effects of bracing on the proprioceptive abilities of the ankle joint, limiting other cutaneous activation of the foot, by computer modeling the movement of the ankle through 3-Dimensional space. Testing of non-athlete controls and elite volleyball athletes from various collegiate programs is currently underway. Using the XSENS MTw™ motion sensor system (Enschede, The Netherlands), subjects are asked to visually guide their foot at the ankle joint as they touch their hallus to a target positioned medially and inferiorly to the first metatarsophalangeal joint. Trials are repeated under two further

conditions: first, subjects are blindfolded; second, subjects are blindfolded while wearing an orthoses on the ankle complex being examined. By utilizing XSENS system in this manner, we will draw comparisons regarding a subject's proprioceptive acuity in the ankle-foot complex, and investigate the role of bracing on proprioception. We anticipate results to demonstrate enhanced proprioceptive ability when wearing the ankle brace in all subject groups. Additionally, bracing will prove more beneficial in athletes with FAI. At this time, further data collection and analysis are required.

161

Determination of Cancer Susceptibility in Probands with Breast and Ovarian Cancer

K.A. Schrader[1], **K.N. Maxwell[2], J.Vijai[1], S. Hart[3], T. Thomas[1], B. Wubbenhorst[2], L. Guidugli[3], R. Klein[1], M. Corines[1], L. Zhang[1], S. Neuhausen[4], J. Weitzel[4], N. Gupta[5], L.Norton[1], C. Hudis[1], G. Getz[5], M. Daly[5], S. Lipkin[6], D. Altshuler[5], F. Couch[3], K. Nathanson[2], K. Offit[1]**

[1]Memorial Sloan-Kettering Cancer Center, New York, NY; [2] University of Pennsylvania, Philadelphia, PA; [3]Mayo Clinic, Rochester, MN; [4]Beckman Research Institute of City of Hope, Duarte, CA; [5]Broad Institute, Boston, MA; [6]Weill Cornell College of Medicine, New York, NY

Genome wide association studies show little overlap between common variants associated with breast and ovarian cancer susceptibility. However, germline susceptibility to breast and ovarian cancers can be seen in association with rare mutations of *BRCA1/2*. Furthermore rare variants in other genes initially identified as breast cancer susceptibility genes also confer susceptibility to ovarian cancer cases, unselected for breast cancer. To further elucidate rare germline susceptibilities for both breast and ovarian cancer, we have ascertained a unique cohort of *BRCA1/2*-negative probands with a personal history of breast cancer and ovarian/fallopian tube/peritoneal cancer, unselected for family history (90 cases). Cases are from the clinical genetics services of Memorial Sloan-Kettering Cancer Center and University of Pennsylvania. Analysis is ongoing using both targeted and exome sequencing approaches. Analysis of variants is being undertaken across and within histologic subtypes to look for specific genotype-phenotype correlations. Validation of candidate novel susceptibility genes and pathways by co-segregation, tumor studies, functional analysis, as well as external replication in corresponding phenotypic cohorts to determine whether shared rare variants, mutated genes, or altered pathways confer cancer susceptibility in this cohort is underway. Germline susceptibility to breast and ovarian cancer is heterogenous and remains a critical research and clinical question. Improved understanding of the susceptibility genetics will allow further examination of the utility of targeted cancer prevention strategies in those who are found to be at risk.

162

Novel Malaria Transmission Blocking Vaccines

Lukasz J. Sewera[1], **Kathleen A. Mcguire[1], Kim C. Williamson[2] and Christopher M. Wiethoff[1]**

[1]Department of Microbiology and Immunology, Loyola University Chicago Stritch School of Medicine, Maywood IL; [2]Department of Biology, Loyola University Chicago, Chicago IL

Eradication of Plasmodium spp., the causative agent of Malaria, requires a multifaceted approach. Vaccines targeting pre-erythrocytic and erythrocytic stages as well as vaccines that block transmission by the mosquito vector are a key component of eradication efforts. The sexual stage antigen, P25, is required for parasite invasion of the mosquito midgut and strong evidence supports the ability of anti-P25 antibodies to prevent transmission via mosquito vectors. A major limitation to the induction of transmission blocking anti-P25 antibodies is the apparently poor immuno-stimulatory properties of the recombinant P25 protein. Using the P25 antigen from Plasmodium falciparum, Pfs25, we have developed adenovirus-based vaccine vectors that express an optimized Pfs25 transgene or display 720 copies of putative Pfs25 B-cell epitopes in the hexon protein of human adenovirus 5 (Ad5). Intramuscular priming with replication incompetent Ad5 vectors expressing Pfs25, Ad5pfs25, elicits significantly greater IgG responses to Pfs25 than yeast produced Pfs25/Alhydrogel. Pfs25 epitopes from the DII and DIII EGF-like domains are recognized by anti-Pfs25 serum when displayed in several hypervariable regions of the Ad5 hexon. Further, secondary immunization of mice primed with Ad5pfs25 usingAd5 capsid displayed Pfs25 epitopes elicits strong Pfs25 specific IgG responses capable of binding P. falciparum parasites. However, ELISA data demonstrate that Ad5pfs25 prime-Pfs25/Alhydrogel boost elicits the greatest IgG response.

164

Inhibition of the Non-Gastric H^+/K^+ ATPase Increases pH of Cystic Fibrosis Airway Surface Liquid

VS Shah, **SE Ernst, CO Randak, and MJ Welsh**

University of Iowa, Iowa City, IA

Cystic Fibrosis (CF) is a disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which encodes an anion channel that transports of Cl^- and HCO_3^- across epithelial cells. The predominant cause of morbidity and mortality in CF is lung disease. Previously, we disrupted the CFTR gene to generate a porcine CF model. At birth, CF pigs have a host defense defect, and they spontaneously develop lung disease similar to humans with CF. We recently discovered that CF airway surface liquid (ASL), the thin layer of fluid on the surface of airway epithelia, has defective bacterial killing due to an abnormally acidic pH. Alkalinizing the ASL in CF pigs rescued this defect. To better understand the cellular and molecular mechanisms that control ASL pH, we studied H^+ and HCO_3^- transport into the ASL. We measured ASL pH in primary cultures of porcine airway epithelia using a pH sensitive fluorophore and found a reduced ASL pH in CF ASL. We treated cultures with forskolin (10μM) and IBMX (100μM) to increase

intracellular cAMP and stimulate CFTR. In non-CF epithelia, increasing cAMP stimulated HCO_3^- secretion, consistent with CFTR activity. However, cAMP failed to stimulate HCO_3^- secretion in CF epithelia, and instead decreased ASL pH, suggesting an increased H^+ secretion. To learn whether non-CF epithelia also secrete H^+, we eliminated the effects of HCO_3^- secretion by using HCO_3^- free solutions and found that ASL pH became more acidic with forskolin and IBMX. Using RT-PCR, we found mRNA expression of a number of potential H^+ transporters in airway epithelia. We then pharmacologically inhibited these proteins and found that ouabain, which inhibits the non-gastric H^+/K^+ ATPase, increased ASL pH. To more specifically inhibit the non-gastric H^+/K^+ ATPase, we developed an siRNA to the alpha subunit, ATP12a, and found that knockdown of ATP12a decreased ASL acidification and reduced forskolin and IBMX dependent acidification. In CF epithelia, 10μM apical ouabain increased ASL pH by ~0.2 pH units. To test if the increase in pH might have physiologic effects, we quantified the effect on antimicrobial defense by measuring ASL killing of *Staphalococcus aureus*, a common cause of airway infection. The percent bacteria killed increased from 26% to 44% upon inhibition of the non-gastric H^+/K^+ ATPase. These results indicate that the non-gastric H^+/K^+ ATPase contributes to ASL pH, and is stimulated by forskolin/IBMX. Inhibiting this transporter increases ASL pH and consequently increases bacterial killing. Thus, our results suggest that inhibiting the non-gastric H^+/K^+ ATPase in CF may be of therapeutic benefit.

165

Inflammatory Protease, Caspase-1, is Released from Monocytes in a Stably Active Form in Response to Endotoxin

Shamaa, OR*, Wewers, MD

**Medical Scientist Training Program,*Biomedical Sciences Graduate Program, Davis Heart and Lung Research Institute, Division of Pulmonary, Allergy, Critical Care and Sleep Medicine, Department of Internal Medicine, Wexner Medical Center, The Ohio State University, Columbus, OH*

Introduction: Caspase-1 is an inflammatory protease, which is part of the cysteine-aspartate protease (caspase) family, and is required for cleavage of the acute pro-inflammatory cytokines, interleukin-1β (IL-1β) and IL-18, as well as for a form of cell death termed pyroptosis. Caspase-1 is synthesized as a zymogen which requires the assembly of a multi-protein complex, termed the inflammasome, for its activation. This assembly forms in response to pathogen or danger associated molecular patterns, triggering an acute inflammatory response, but where and how this assembly happens in the cell is poorly understood. To address this question we compared the activity of caspase-1 in the released fraction as compared to the cytosolic form in the human monocyte cell line, THP-1. **Results:** In response to endotoxin, pro-caspase-1 is rapidly processed and released from monocytes in its mature, p20/p10, form. We found that the released form has activity (4.2 ±1.3 AFU/min) by WEHD-afc cleavage which is stable for over 12h and this activity is inhibited by the tetra-peptide caspase-1 specific inhibitor (YVAD-cmk). Released activity was enhanced in the presence of serum in the media. Interestingly, however we were unable to completely immunodeplete the released caspase-1 activity or show its ability to cleave exogenously added proIL-1β. This contrasts to the *in-vitro* cell-extract model, where concentrating monocytic lysates to 10μg/μl spontaneously activates caspase-1. This cytosolic caspase-1 cleaves endogenous proIL-18 but rapidly loses function, with a $t_{1/2}$≈12 min. **Conclusions:** These results suggest that caspase-1 activation requires a multi-level regulation that links assembly of the inflammasome complex and enzymatic activity to its interaction with its target substrates during release. Understanding these differences between the released and cytosolic inflammasomes provides a novel opportunity to gain fundamental insight into the details that regulate the acute inflammatory response.

166

A Novel Biocompatible Polymeric Artificial Iris Design

***F Shareef*, S Sun, M Kotecha, D Azar, M Cho**

University of Illinois at Chicago, Chicago, IL

Purpose: Patients with permanent iris damage suffer from excessive light exposure leading to photophobia, glare and poor vision. Current artificial irises lack the dynamic response to light inherent in the natural iris. A new artificial iris that attenuates light based on intensity and wavelength was developed using a combination of a photo-responsive material encased in a biocompatible polymer matrix. Photo-responsive materials are activated by UV/visible light to change opacity and decrease light transmission. **Methods:** Photopia, a photo-responsive material in polyethylene (Matsui International), was shaped into annular disks of 12mm outer diameter, 3mm inner diameter, 150μm thickness and spin-coated with a 100μm layers of polydimethylsiloxane (PDMS, Sylgard 184, Sigma) on either side to form the artificial iris. Wavelength scans (100-1100nm) of percent light transmitted were obtained using a UV/Visible spectrophotometer. Human corneal fibroblasts (HCFs) exposed to the artificial iris for 1 day, 2 weeks, and 1 month were stained with live/dead cell viability assay and imaged using a spinning disk confocal microscope. Photopia and the artificial iris in PBS were tested using NMR to explore potential leeching. **Results:** Optical testing of the artificial iris showed activation by UV and blue light in 5 seconds and complete reversal in 1 minute. Wavelength scans determined 40% of visible and 60% of UV light was blocked. Quantification of live HCFs exposed to Photopia alone showed 30% cell death after 2 weeks. However, HCFs in contact with the artificial iris had no cell death similar to control samples. NMR results showed no apparent leakage of toxic substances from the artificial iris. **Conclusion:** Incorporating a photo-responsive material in a polymer matrix provides a novel artificial iris design. By actively attenuating incident bright light, our device better mimics the natural iris. In vitro biocompatibility tests established Photopia alone may be toxic to cells but encasing in PDMS to make the artificial iris serves as a barrier effectively preventing cell death. NMR results confirmed these findings. Thus, our artificial iris may provide a new treatment option for patients with permanent iris damage.

Poster Abstracts

167

Role for T-Cell Specific Adapter Protein (TSAd) in Alloimmunity

MM Sheehan, **MP Stack, T Seto, K Liu, N Kochupurakkal, and DM Briscoe**

Transplant Research Program, Boston Children's Hospital, Harvard Medical School, Boston, MA

Long-term success following organ transplantation requires an understanding of the alloimmune response and events leading to immunoregulation. TSAd is an SH2 domain containing intracellular adapter molecule that functions to elicit T cell receptor-mediated signals resulting in cytokine production. TSAd knockout (KO) T cells exhibit reduced proliferation in response to mitogen stimulation and secrete reduced quantities of IL-2, IL-4 and IFN-γ. Here, minor MHC class II mismatched B6.C-H-2^{bm12} donor hearts were transplanted into wild type (WT) C57BL/6 (H-2^{b}) or TSAd KO (H-2^{b}) recipients. While the median survival time (MST) for grafts in WT recipients was >45 days, contrary to our expectation, graft failure was markedly accelerated (MST=22 days, P<0.005, n=10) in TSAd KO recipients. We also performed fully mismatched BALB/c (H-2^{d}) donor heart transplants into WT or TSAd KO recipients. Again, contrary to our expectation, TSAd KO recipients (MST= 8 days, n=5) did not have a survival advantage in comparison to WT recipients (P=NS). We treated fully mismatched recipients with anti-CD40L (200 mcg on day 0 and day 2) to inhibit effector T cell (Teff) expansion, and, while graft survival in WT recipients was >30 days, all grafts in TSAd KO mice failed by day 21 post-transplantation (n=6, P <0.005). By FACS, we found increased numbers of Teffs (n=3, P<0.001) and increased IFN-γ □□□□□□□□□□□□□□□□□□(□=3,□=0.05)□□□□□□□□□□□□□□□□□□□□□□□□ BALB/c transplant model. In addition, when naïve or alloprimed $CD4^{+}CD25^{-}$ T cells were cocultured with $CD4^{+}CD25^{+}$ regulatory T cells (Tregs) from WT or TSAd KO recipients, we found that the suppressive function of TSAd KO Tregs was reduced in comparison to WT Tregs. To evaluate whether TSAd KO mice exhibit an inability to expand induced Tregs (iTregs), we next cultured $CD4^{+}CD25^{-}$ T cells (WT or TSAd KO) with mitogen (anti-CD3/CD28), IL-2, TGF□ and either rapamycin or retinoic acid. We observed similar numbers of $CD4^{+}CD25^{hi}Foxp3^{+}$cells in both WT and TSAd KO mice. Interestingly, however, in pilot experiments, these TSAd KO $Foxp3^{+}$ T cells failed to suppress Teffs as efficiently as WT Tregs. These findings suggest that TSAd signaling is critical to generate functional iTregs *in vivo*. Furthermore, these findings suggest that reduced IL-2 production by TSAd KO Teffs does not account for this functional deficiency. Collectively, these findings, for the first time, identify TSAd as a critical mediator of immunoregulation following allograft transplantation.

168

Transcriptional Control of Human Cellular Metabolism by Wasp Venom: *Nasonia vitripennis* Venom-Derived Bioactive Peptides as Candidate Therapeutics for Human Metabolic Disorders

AL Siebert, **PJ Simpson-Haidaris, JH Werren**

University of Rochester, School of Medicine and Dentistry, Rochester NY

Background: Animal venoms are known sources of biological components with therapeutic value. An example of a successful venom-based drug is captopril used in the treatment of hypertension. We now aim to explore parasitoid wasp venoms for medical applications. Parasitoid wasps are an immense group (>100,000 species) whose venoms have been underexplored for drug discovery. Parasitoid venoms are functionally distinct from those of other venomous animals in which venom is used for defense against predators, immobilization of prey, and/or digestion. In contract, parasitoid venoms arrest normal physiology and manipulate conserved metabolic pathways to optimize the balance of lipids, amino acids, and fats. During this process the host remains alive and transcriptionally active for days. The venoms of parasitoid wasps are likely to contain metabolic drug candidates quite different from venoms previously studied (e.g. snakes, cone snails, spiders), which mainly target coagulation and neurophysiology. **Methods:** Illumina Hi-Seq analysis of total RNA extracted from human renal mesangial cells (HRMCs) dosed with a wide range of venom concentrations (128 – 1/128 venom reservoir equivalents per mL of media) and across three time points (1h, 4h, 24h). Transcriptomic data was analyzed by CuffDiff analysis as well as Limma (for RNA Seq) to identify dose and time-series trends. **Results:** Initial experiments in human cell culture demonstrate metabolic effects of parasitoid venom. Our study organism is the model parasitoid *Nasonia vitripennis* (*NV*). We previously found that *NV* venoms specifically target sugar, lipid, pyruvate, and amino acid metabolism in fly hosts (*Sarcophaga bullata*). High dose venom was lethal to human cells. Low dose venom causes gene expression changes, which aggregate in sugar metabolism and steroid hormone biosynthetic pathways and appear to stimulate cell growth. These data demonstrate that *NV* venom can specifically regulate human gene transcription. **Future directions:** We now aim to determine in which ways parasitoid venoms alter metabolism in human cells, and to identify candidate small molecules and secreted peptides with metabolic effects relevant to the study and treatment of human disease.

169

Constitutively STIM(1)ulated Calcium Entry in Skeletal Muscle

Kavisha Singh, **Victoria Bryson, Elizabeth Finch, Tianyu Li, Paul Rosenberg**

Duke University Medical Center, Durham, NC

Background: STIM1 plays a key role in mediating Store Operated Calcium Entry (SOCE), which is a critical mechanism in the development and function of excitable tissues such as skeletal muscle. In this study, we use a mouse model with an EF hand mutant (D84G) of STIM1 (SAX) that works as a gain of function model and results in pre-activation of STIM1 to cause

constitutive SOCE. **Materials and Methods:** We used various techniques to characterize the function and calcium signaling in the SAX mouse model. Muscle histology, western blotting, calcium imaging, field stimulation experiments and behavioral studies were conducted to study the mouse model. **Results:** Our studies showed that SAX mice had increased presence of central nuclei in their skeletal muscle, suggesting a pathology that was also verified by their decreased activity end point measures in the behavioral experiments, such as reduced open field activity and increased number of foot faults/cm moved. The behavioral studies also showed an abnormal response to stress, with markedly reduced vertical open field activity in the SAX mice post exercise. Ca imaging and field stimulation experiments showed that basal calcium levels were elevated in SAX myofibers and they underwent a quick calcium buildup with a subsequent decrement in the amplitude of the pulses on repeated electrical stimulation. **Conclusion:** Our results provide evidence that the D84G EF hand mutation in STIM1 causes alterations in calcium homeostasis, which is seen in the elevated basal Ca2+ levels of SAX myofibers and the fast calcium buildup and fatigability of the fibers when subjected to repeated field stimulation. Our in vivo behavioral studies and the presence of central nuclei also support the idea that the SAX mouse line has a myopathy that is age dependent, although the presence of tubular aggregates remains unverified. By conducting the future studies mentioned above, we hope to gain further insight into the role of SOCE in the function and development of skeletal muscle.

170

Controlling the Intracellular Delivery of Spherical Nucleic Acids by Engineering Oligonucleotide Sequence

S Narayan, **C Choi, L Hao, C Calabrese, E Auyeung, C Mirkin**
Northwestern University, Evanston, IL

Intracellular delivery of nucleic acids for gene regulation typically requires the use of cationic carriers, but cytotoxicity associated with such carriers reduces their attractiveness for therapeutic use. Spherical nucleic acids (SNAs), consisting of densely packed, highly oriented oligonucleotide strands attached to the surface of nanoparticles, are able to overcome the typical challenges of nucleic acid delivery. SNAs have been shown to effectively enter 50 different cell types without the use of auxiliary transfection agents and exhibit minimal cytotoxicity. Recently, the mechanism of endocytosis of these polyanionic structures was shown to be dependent on class A scavenger receptors, which allow for uptake of SNAs into cells through lipid raft-mediated endocytosis. Linear poly(guanosine) DNA strands are natural ligands for class A scavenger receptors, whereas DNA strands composed of adenosine (A), thymidine (T), or cytosine (C) are non-ligands. We expect that exploiting the natural interactions of class A scavenger receptors with poly(guanosine) oligonucleotide strands, by constructing SNAs whose constituent oligonucleotide strands are rich in guanosine (G), will maximize the uptake of SNAs into cells. In this work, we show that G-rich SNAs exhibit several-fold higher uptake into cells than SNAs rich in A, T, and C. The phenomenon of increased intracellular delivery can be explained, at least partly, by an increased affinity of class A scavenger receptors for G-rich SNAs; measurements of binding affinity show that G-rich SNAs exhibit stronger binding to class A scavenger receptors than SNAs rich in A, T, and C. This work presents an effective strategy to maximize the intracellular delivery of SNAs, which is potentially applicable to other nanoparticle systems, thus establishing an important design consideration for nanoparticle-based intracellular delivery of therapeutics.

171

Comparative Efficiency of HIV-1-Infected T Cell Killing by NK Cells, Monocytes, and Neutrophils

Adjoa Smalls-Mantey*,* **Mark Connors and Quentin J Sattentau**
**The Sir William Dunn School of Pathology, University of Oxford, Oxford, UK*

Background: HIV-1 infected cells are eliminated in infected individuals by a variety of cellular mechanisms, the best characterized of which are cytotoxic T cell and NK cell killing. An additional antiviral mechanism is antibody-dependent cellular cytotoxicity mediated by NK cells (NKs) and monocytes (MCs). Neutrophils (PMNs), classically considered as antibacterial cells have also been shown to exhibit anti-HIV-1 activity but the relative cytotoxicity of these effector cells is yet to be characterized. **Methods:** Primary $CD4^+$ T cells were infected with $HIV\text{-}1_{BaL}$ and used as target cells. Autologous NKs, MCs, and PMNs were used as effectors and infected target cell killing by effectors was measured by flow cytometry. This was carried out in the presence or absence of HIV-1-specific antisera to assess antibody-dependent or intrinsic mechanisms of cellular cytotoxicity. **Results:** At the same effector to target ratio, NKs and MCs mediated similar levels of both antibody-dependent and antibody-independent killing of HIV-1-infected T cells. PMNs also killed infected T cells via intrinsic mechanisms and mediated significant antibody-dependent killing of T cells, but were less effective than NKs or MCs. Laser-scanning confocal microscopy confirmed effector cell induced apoptosis and revealed that MCs rapidly ingest apoptotic bodies of killed cells. **Conclusion:** These data have implications for acquisition and control of early HIV-1 infection by PMNs and MCs prior to activation of an adaptive immune response, at later stages in the presence Env-specific antibodies, and is relevant to vaccine-induced anti-HIV-1 antibody-based effector mechanisms.

172

Analysis of > 300,000 Individuals Gives New Insights into the Genetics and Biology of Body Mass Index

Elizabeth Speliotes
GIANT Consortium, University of Michigan, Ann Arbor, MI

Obesity is a heritable condition that affects about a third of the U.S. population. Obesity predisposes to development of metabolic disease but has few effective treatments. We conducted the largest genome-wide meta-analysis of SNP associations with body mass index (BMI), the most common measure of obesity, combining data from up to 339,224 individuals from 125 studies. We confirmed 41 established obesity loci and identified 56 new loci associated with BMI ($P < 5x10^{-8}$). Polygene analysis

indicates that substantial additional heritability is captured by SNPs below the threshold of genome-wide significance. We use novel methods to integrate eQTL, functional variant, literature connection, gene expression, protein-protein interaction, mouse knockout phenotype, and pathway database information at associated loci. Our data confirm a prominent role for central nervous system regulation of BMI, and extend previous work by newly implicating additional biological processes as regulators of human body mass. These include CNS processes such as synaptic function, cell-cell adhesion and glutamate signaling (*PCDH9*, *CADM2*, *NRXN3*, *NEGR1*, *GRID1*), as well as peptide biology (*GRP*, *SCG3*), lipid metabolism (*NPC1*, *DGKG*), and glucose/insulin action (*RPTOR*, *FOXO3*, *TCF7L2, GIPR, IRS1*). Of note, one of the proposed mechanisms of topiramate, a component of a recently approved anti-obesity drug is an effect on CNS glutamate signaling, supporting this and other pathways that we identify as possible targets for obesity intervention. Pleiotropy analysis identified a shared genetic etiology between BMI and related metabolic disease, which may help explain why some but not all obese individuals develop metabolic disease. These results greatly enhance our understanding of BMI biology, open the door for further research into the etiology of obesity and to developing new ways to prevent and treat obesity and its complications.

174

Contextual Synthetic Lethality in Human Triple Negative Breast Cancer Cells Involving Novel Interaction Between DNA Repair Proteins EGFR, PARP1, and BRCA1

***Stanley JA*[a], Nowsheen S[a], Cooper T[a], Forero, A[a], LoBuglio, AF[a], Yang ES[a]**

University of Alabama at Birmingham Comprehensive Cancer Center, Birmingham, AL

Background: Few therapeutic options are effective for triple negative breast cancers (TNBCs) necessitating novel approaches. We previously reported a novel synthetic lethality with combined EGFR and PARP inhibition due to a homologous recombination (HR) repair defect induced EGFR inhibition. The mechanism of the HR deficiency involved disruption of EGFR/BRCA1 interaction and sequestration to the cytosol. In this study, we extended these findings to encompass multiple subtypes of TNBC and EGFR inhibitors. Additionally, we investigated the mechanisms by which EGFR regulates HR DNA repair. **Methods:** TNBC cells, including MDA-MB-231 (mesenchymal stem-like), MDA-MB-468 (basal-like), HCC1187 (immunomodulatory), BT549 (mesenchymal), MDA-MB-453 (luminal androgen receptor), were assessed for cytotoxicity and cell viability following EGFR inhibition (lapatinib, erlotinib, or cetuximab), PARP inhibition, or both by colony formation or ATPlite assays. MALDI-TOF mass spectroscopy was utilized to ascertain protein-protein interaction. To confirm interaction data and determine subcellular location we utilized immunoprecipitation and subcellular fractionation. To determine if interactions were dependent on DNA, lysates were treated with Ethidium Bromide and DNAse prior to immunoprecipitation. **Results:** EGFR inhibition with lapatinib, cetuximab, or erlotinib induced a contextual synthetic lethality with the PARP inhibitor, veliparib, *in vitro* in multiple TNBC subtypes. Interestingly, mass spectroscopy reaffirmed EGFR interaction with BRCA1 but also identified a novel interaction between EGFR and PARP1. These interactions were not found upon EGFR inhibition with lapatinib. Furthermore, we validated with immunoprecipitation and *in vitro* protein binding assays EGFR, BRCA1, and PARP1 protein-protein interactions. Additionally, these proteins interact in the nucleus, independent of DNA. Lastly, EGFR inhibition reduced nuclear EGFR and BRCA1 without changes in PARP1 localization. **Conclusions:** These results reveal a contextual synthetic lethality between combined EGFR and PARP inhibition in multiple subtypes of TNBC, suggesting potential generalizability of this combination in patients with TNBC. Furthermore understanding the mechanism by which EGFR regulates DNA repair may provide novel targets to confer other contextual synthetic lethalities that may be exploited to treat the aggressive TNBCs.

175

Clinical Translation of the Calcium Hypothesis of Cardiomyopathy in Duchenne Muscular Dystrophy

***Su JA*[1], Ramos-Platt L[2], Menteer J[1]**

[1]Division of Cardiology, Department of Pediatrics, Children's Hospital Los Angeles, Los Angeles, CA; [2]Division of Neurology, Department of Pediatrics, Children's Hospital Los Angeles, Los Angeles, CA

Background: Dilated cardiomyopathy (DCM) inevitably afflicts patients with Duchenne muscular dystrophy (DMD). Understanding of the molecular pathophysiology of DCM in DMD has advanced, but correlation of these advances with clinical disease is lacking. The "calcium hypothesis" of DMD disease progression suggests that damage to the cardiomyocyte membrane structure leads to inappropriate influx of calcium ions into cells, which eventually becomes toxic. We sought to identify echocardiographic changes that occur due to calcium dysregulation prior to development of overt DCM in patients with DMD. **Methods**: We retrospectively examined 265 serial echocardiograms from 73 patients with confirmed DMD (ages 0 to 21). We measured fractional shortening (LVSF %), velocity of circumferential fiber shortening (VCFc), indexed left ventricular (LV) mass, and indexed left ventricular internal dimension in diastole (LVIDdi). For a portion of the analysis, patients were stratified by age group (0 to 5, 6 to 10, 11 to 15, and 16 to 21 years), and echocardiographic differences between the groups were analyzed with ANOVA and Student's t-testing. **Results**: As a group, patients aged 11 to 15 years old displayed a decrease in LVIDdi (Z = -1.5) compared with 6 to 11 year olds (Z = -0.5, P < 0.0007) and 0 to 5 year olds (Z = -0.1, P < 0.0001). On average, LVIDdi Z-score was reduced by 0.92 (95% CI 0.4 to 1.45) SD for 11 to 15 year olds versus 6 to 10 year olds. The effect was independent of LV mass, LVSF %, and VCFc. Tonic contraction (LVIDdi Z-score < -2) was observed in patients at a median age of 13 years (range 8 to 21, SD 3.63 years), and was not related to ventricular hypertrophy. In our cohort, no child developed overt ventricular dilation (LVIDdi Z-score > +2) prior to 16 years of age. **Conclusions**: Reduction in LV internal dimension during the second decade of life, before ventricular dilation occurs, is a common finding in DMD patients and appears to be a result of tonic contraction of the LV during the early phase of DCM evolution, not due to ventricular hypertrophy. This phenomenon may be a clinical correlate of the "calcium hypothesis" of DMD physiology in which calcium efflux capacity is overwhelmed by

ion leaks in the cell membrane, leading to abnormally elevated myocardial tone prior to cardiomyocyte apoptosis, myocardial fibrosis, and eventual overt DCM in patients with DMD. Echocardiographic identification of tonic ventricular contraction is thus a promising biomarker for early detection of cardiomyopathy in DMD.

176

Myeloid Dendritic Cells Regulate Hematopoietic Stem and Progenitor Cell Trafficking in the Bone Marrow

***T Supakorndej*[1,2], M Rao[1,2], and DC Link[1]**

[1]Department of Internal Medicine, Washington University in St. Louis, St. Louis, MO; [2]MD-PhD Program, Washington University in St. Louis, St. Louis, MO

Hematopoietic stem and progenitor cells (HSPCs) are found in perivascular niches within the bone marrow, where niche-supportive factors such as CXCL12 can be found. Granulocyte-colony stimulating factor (G-CSF) is the prototypic agent used to mobilize HSPCs into the blood where they can then be harvested for stem cell transplantation. How G-CSF affects these niches to mobilize HSPCs is poorly understood. Moreover, it is believed that leukemic cells may occupy these niches and disrupt normal HSPC function, adding to the need to understand the biology of the bone marrow microenvironment. We have found that G-CSF signaling in a $CD68^+$ monocyte/macrophage lineage cell within the bone marrow initiates the HSPC mobilization cascade. CD68 marks a heterogeneous cell population that includes monocytes, macrophages, myeloid dendritic cells, and osteoclasts. We found that G-CSF suppresses monocyte, macrophage, and myeloid dendritic cell (MDC) numbers in the bone marrow. Bone marrow MDCs are found perivascularly and in close association with stromal CXCL12-abundant reticular cells, suggesting a role for MDCs in maintaining HSPC niche function. To determine the role of MDCs in affecting HSPC retention in the bone marrow, we used transgenic mice expressing the diphtheria toxin receptor under the control of the Zbtb46 promoter (Zbtb46-DTR) to specifically target MDCs. We found that Zbtb46 is expressed in MDCs but not monocytes nor macrophages. Zbtb46-DTR mediated MDC ablation resulted in significant mobilization of HSPCs into the blood and spleen. Moreover, MDC ablation enhanced mobilization of these cells by G-CSF, providing evidence that MDCs contribute to G-CSF-induced HSPC mobilization. G-CSF mobilizes HSPCs, at least in part, by decreasing CXCL12 expression in bone marrow stromal cells. CXCL12 expression from these cells is required for HSPC maintenance. We found that MDC ablation also suppresses CXCL12 expression in the bone marrow, suggesting that MDC-derived signals contribute to HSPC maintenance by modulating stromal cells that comprise the perivascular niche.

177

Persistent Responses in Neurons of Perirhinal and Lateral Entorhinal Cortices: A Mechanism for Long-Term Recall of Trace Conditioning

***E. E. Suter,* C. Weiss, J. F. Disterhoft**

Department of Physiology, Feinberg School of Medicine, Northwestern University, Chicago, IL

Perirhinal (PR) and lateral entorhinal (latEC) cortices, major bidirectional connecting nodes between higher-order cortex and hippocampus, contain neurons that show persistent activity in vitro following a brief stimulus, suggesting that these parahippocampal areas may possess mechanisms for bridging time between associated stimuli in vivo. We have recently shown that PR, but not latEC, is essential for acquisition of trace eyeblink conditioning (tEBC), in which a 500ms gap (trace period) separated conditioned (CS; whisker vibration) and unconditioned stimuli (US; corneal airpuff). LatEC is however required for retention of trace EBC. To understand the functional role of neurons in each of these areas, we placed tetrodes into the PR, latEC and hippocampus and then recorded single-neuron activity in the awake rabbit during trace EBC acquisition and long-term retention testing. Among 408 cells recorded in PR and latEC, significant rate modulation (a statistically significant increase or decrease in peri-stimulus firing rate) was seen in 44% of neurons during task acquisition. One month after acquisition training, rabbits were tested for consolidated retention of tEBC. During this post-consolidation time period, 65% of neurons exhibited significant rate modulation, suggesting that these parahippocampal areas remain involved in long-term recall of trace conditioning. Trace-period rate modulation (significant rate modulation between the CS and US) was seen in similar numbers of rate-increasing (9%) and rate-decreasing (10%) neurons in PR and latEC during acquisition. In contrast, rate-decreasing trace-period responsive neurons were more prevalent post-consolidation at 25% of neurons, while rate-increasing responses remained at 10%. These results suggest that rate-decreasing cells are more prevalent post-consolidation than during acquisition in PR and latEC, possibly representing an increased inhibitory component in the circuit and/or a mechanism for signal-to-noise improvement. We conclude that persistent post-stimulus responses in PR and latEC may contribute to binding stimuli together across time, and may support long-term recall of trace conditioning. Further single-neuron recording data are needed to clarify the role of rate-decreasing and rate-increasing neurons pre- and post-consolidation and to draw conclusions about the network function of these temporal lobe structures in long-term memory.

178

Characterization of Hypoxia Signaling in Bladder Cancer

***PJ Sweigert,* KE Foreman, GN Gupta**

Loyola University Chicago Stritch School of Medicine, Maywood, IL

Introduction and Objectives: Hypoxia is a common feature of solid tumors and can induce a cascade of tumor glycolysis, angiogenesis and other cell survival responses by activating transcription through hypoxia inducible factors (HIFs). HIFs are transcription factors that are constitutively expressed and tightly

regulated in an oxygen dependent manner. Under normoxic conditions, HIFα is rapidly ubiquitinated and targeted for destruction by the proteosome. Yet, under hypoxic conditions, HIFα is stabilized and translocates to the nucleus where it promotes transcription of various genes required for angiogenesis (VEGF), glucose transport (Glut1), glycolysis (lactate dehydrogenase) and erythropoiesis (erythropoietin). Previous reports suggest HIFα is aberrantly expressed in bladder cancers even under physiologic oxygen concentrations. Aberrant hypoxia signaling is considered a significant tumor–promoting event, and we examined HIFα expression and hypoxia signaling in a panel of bladder cancer cell lines ranging from superficial to invasive disease. **Methods:** RT4, SW780, HT1376, J82, UMUC3 and T24 bladder cancer cell lines were cultured under standard, normoxic conditions. Western blot was performed using nuclear protein extracts and antibodies directed against HIF1α and HIF2α. HIF1α transcriptional activity was assessed using an ELISA that measures binding of HIF to an oligonucleotide containing a hypoxia response element (HRE) oligonucleotide sequence. VEGF secretion was measured in cell culture media using an ELISA, with colorimetric absorbance values normalized to cell count. **Results:** HIF1α and HIF2α protein expression was readily detectable in bladder cancer cell lines, but not normal cultured urothelial cells as determined by Western blot. HIF transcriptional activity was demonstrated through binding of HIF to a HRE that could be blocked by competitive binding of a specific oligonucleotide. The results were confirmed as VEGF, a downstream target of HIF signaling, was detected in the media of bladder cancer cell lines. **Conclusions:** Identification of transcriptionally active HIFα in bladder cancer cell lines cultured in normoxia, but not normal urothelial cells, provides evidence for aberrant hypoxia signaling in bladder cancer. Development of novel therapeutics targeting hypoxia signaling may be of therapeutic benefit patients to patients with advanced bladder cancer.

179

Sphingosine-1-Phosphate Receptor 2 is Elevated in Patients with Pulmonary Arterial Hypertension and Regulates Pulmonary Artery Smooth Muscle Cell Proliferation

***JR Sysol*[*,†], J Chen[*], H Tang[*], JX-J Yuan[*,†], V Natarajan[*,†], RF Machado[*,†]**

[*]Department of Medicine, Section of Pulmonary, Critical Care Medicine, Sleep and Allergy, [†]Department of Pharmacology, University of Illinois at Chicago, Chicago, IL

Background: Sphingosine-1-phosphate (S1P), a bioactive sphingolipid, is involved in modulating cell proliferation and survival via binding to a family of five G-protein coupled receptors, known as S1PR1-5. S1P is elevated in the lungs of patients with pulmonary arterial hypertension (PAH), potentially playing a role in the pathologic pulmonary vascular remodeling in this disease. In this study, we aimed to investigate whether S1P mediates PASMC proliferation through specific receptor ligation. **Methods**: We first measured S1PR expression in PASMCs from PAH patients (n = 5) and controls (n = 4) via Western blotting. S1PR2 expression was elevated in PAH patients, so this receptor was analyzed in subsequent studies. Next, BrdU incorporation was used to measure S1P-induced proliferation in normal human PASMCs. Cells were stimulated with either S1P (100nM and 1μM) or media for 24h. To determine the effect of silencing S1PR2 on S1P-induced human PASMC proliferation, two methods were used: (1) Cells were transfected with either scrambled-siRNA or S1PR2-siRNA before measuring BrdU incorporation. (2) Cells were treated with a S1PR2-specific pharmacologic inhibitor, JTE-013 (10μM), before measuring BrdU incorporation. Additionally, PASMCs were isolated from S1PR2$^{-/-}$ and WT mice. Cell proliferation was measured in these cells via BrdU incorporation at baseline and after both S1P stimulation (100nM and 1μM, 24hr) and hypoxia exposure (3% O_2, 48hr). **Results**: S1PR2 expression was increased in PASMCs from PAH patients. Treatment of human PASMCs with S1P significantly stimulated proliferation. S1PR2-siRNA, but not scrambled-siRNA, decreased basal proliferation and completely prevented S1P-mediated proliferation. Inhibition of S1PR2 with JTE-013 also attenuated basal and S1P-induced proliferation in human PASMCs. In PASMCs isolated from S1PR2$^{-/-}$ mice, both S1P-induced and hypoxia-induced proliferation was attenuated compared to WT littermates. **Conclusion**: These studies demonstrate that S1PR2 is upregulated in patients with PAH and is important for S1P-mediated proliferation of PASMCs, potentially contributing to pulmonary vascular remodeling. Therefore, S1PR2 could be a novel therapeutic target in PAH.

180

Impact of Psoriasis Severity on Hypertension Control: A Population-Based Study in the United Kingdom

***J Takeshita*[1], S Wang[2], DB Shin[1], NN Mehta[3], SE Kimmel[1], DJ Margolis[1], AB Troxel[1], JM Gelfand[1]**

[1]University of Pennsylvania, Philadelphia, PA; [2]Rutgers Robert Wood Johnson School of Medicine, Piscataway, NJ; [3]National Heart Lung and Blood Institute, Bethesda, MD

Psoriasis is a chronic inflammatory disease of the skin that affects 2-3% of the population. More severe psoriasis, in particular, is increasingly recognized as a risk factor for cardiometabolic disease, independent of traditional risk factors (e.g., hypertension) that are known to be more prevalent among psoriasis patients. The effect of comorbid inflammatory diseases such as psoriasis on hypertension is poorly understood. The aim of our study was to assess the impact of psoriasis and its severity as objectively determined by affected body surface area (BSA) on blood pressure control among patients with diagnosed hypertension. We performed a population-based, cross-sectional study using the Incident Health Outcomes and Psoriasis Events (iHOPE) cohort which consists of a random sample of psoriasis patients in The Health Improvement Network (THIN), an electronic medical records database in the UK. In addition to being diagnosed with hypertension, included psoriasis patients (N=1,322) had their psoriasis diagnosis confirmed and psoriasis extent determined by their general practitioner. Up to 10 patients with hypertension and without psoriasis were matched to psoriasis patients by age and practice site (N=11,977). The outcome was uncontrolled hypertension (systolic blood pressure $\geq$ 140 mmHg or diastolic blood pressure $\geq$ 90 mmHg). We found a significant positive dose-response relationship between uncontrolled hypertension and psoriasis severity in both unadjusted and adjusted analyses

that controlled for age, sex, body mass index, smoking and drinking status, presence of diabetes, chronic kidney disease, hyperlipidemia, and cardiovascular disease, and current antihypertensive and non-steroidal anti-inflammatory drug use (adjusted odds ratios [OR] of 0.97, 95% confidence interval [CI], 0.82-1.13 for mild; 1.20, 95% CI, 0.99-1.45 for moderate; and 1.48, 95% CI 1.08-2.04 for severe psoriasis; p for trend=0.01). The greatest likelihood of uncontrolled hypertension was observed among patients with moderate-to-severe psoriasis (≥ 3% affected BSA). Our findings were robust to multiple sensitivity analyses including adjustments for number of blood pressure records and hypertension severity. Our results suggest that patients with concomitant hypertension and psoriasis, particularly those with more severe disease, may require stricter blood pressure management than hypertensive patients without psoriasis.

181

Intestinal Krüppel-Like Factor 4 Reduces Cell Death in the Gut and Contributes to Survival Following Whole-Body Irradiation in Mice

***Daniel Talmasov**+*, Xinjun Zhang[*], Amr M. Ghaleb, Bing Yu, Mandayam O. Nandan, Vincent W. Yang**

*Department of Medicine, Stony Brook University School of Medicine, Stony Brook, NY *These authors contributed equally to this work.*

Background: The zinc finger transcription factor, Krüppellike factor 4 (Klf4), is expressed in differentiated epithelial cells of small intestinal, and is associated with growth arrest. Our previous studies show that Klf4 inhibits apoptosis and induces cell-cycle arrest in irradiated cells *in vitro*. **Aim:** To determine whether intestinal Klf4 contributes mouse survival following whole body irradiation through actions in gut epithelium. **Methods:** Mice with an intestinal epithelial specific deletion of *Klf4* ($Klf4^{\Delta IS}$) and WT mice with floxed *Klf4* gene ($Klf4^{fl/fl}$) were exposed to total body γ-irradiation at 12Gy, and survival was tracked in a timecourse following irradiation. Mice were also sacrificed 6h, 24h, and 96h following irradiation. Immunofluorescence staining for Klf4, the apoptotic marker cleaved caspase3 and the proliferative marker Ki67, and p53 was performed on the small intestine. Number of positively stained cells were counted and irradiated groups were compared to each other and to nonirradiated controls. **Results:** Survivorship was higher in $Klf4^{fl/fl}$ than in $Klf4^{\Delta IS}$ mice. There was significantly greater apoptosis in $Klf4^{\Delta IS}$ mice than in the $Klf4^{fl/fl}$ group at 6 hours, 24 hours, and 96 hours following irradiation. There was also significantly greater p53 expression in $Klf4^{\Delta IS}$ than in $Klf4^{fl/fl}$ mice 6 hours and 24 hours following irradiation. At 96 hours following irradiation, there were slightly more actively cycling cells expressing Ki67 in $Klf4^{fl/fl}$ than in $Klf4^{\Delta IS}$ mice, concurrent with this there was a change in the expression pattern of both Klf4 and Ki67 in $Klf4^{fl/fl}$ mice compared to unirradiated controls, with much a higher number of Klf4/Ki67 double positive cells, and an increased number of Klf4 positive cells in the proliferative zone marked by Ki67 expression. **Conclusion:** Klf4 contributes to mouse survival following total body γ-irradiation, and its absence results in greater apoptosis and greater p53 expression. Klf4 is also associated with the regenerative response of gut epithelium in the days following irradiation.

+Supported in part by an Alpha Omega Alpha Carolyn L. Kuckein Student Research Fellowship.

182

Atopic Allergic Conditions and Colorectal Cancer Risk in the Multiethnic Cohort

***NA Tambe**, L Wilkens, P Wan[*], F Gilliland[*], BE Henderson[*], LL Marchand, CA Haiman[*]**

[*]University of Southern California, Los Angeles, CA; [†]University of Hawaii, Honolulu, HI

Background: Previous studies have shown a potential inverse association between atopic allergic conditions (AAC) and colorectal cancer (CRC) incidence and mortality in predominantly White and female populations. **Methods**: AAC include atopic asthma, allergic rhinitis, and food allergies. We examined whether the association between AAC and CRC differed by ethnic group, location, and stage of cancer in a population-based prospective Multiethnic Cohort (MEC). We also examined potential effect modification when stratified by various associated covariates. There were 4259 incident cases of CRC and 1221 CRC-related mortality cases in the final analysis collected from 1993 to 2010 in ~180,000 White, African American, Native Hawaiian, Japanese American, and Latino men and women in Hawaii and California. **Results:** Overall, individuals with AAC had a reduced risk of colorectal cancer incidence (RR = 0.85, $p < 0.0001$). Significant associations were also seen when stratified by sex and ethnicity, as seen in Whites (RR = 0.84, $p = 0.03$), African Americans (RR = 0.81, $p = 0.01$), and Japanese Americans (RR = 0.85, $p = 0.01$). Individuals with AAC experienced an 18% reduction in risk of regional CRC incidence ($p = 0.0002$). The protective effect of AAC on CRC incidence increased with smoking status (RR = 0.92 in never smokers versus RR = 0.72 in current smokers), and decreased with aspirin status (RR = 0.81 in never users versus RR = 0.94 in current users). In addition, individuals with AAC had a 19% reduction in CRC-related mortality when compared to individuals not suffering from AAC, after adjustment for ethnicity ($p = 0.004$). **Conclusion:** These findings support the inverse association between AAC status and colorectal cancer risk across multiple ethnic populations. The immune surveillance hypothesis is a potential explanation for this inverse association, where atypically increased eosinophilic activity due to AAC leads to anti-tumor activity within the gut. Other possibilities are currently being explored.

183

Human Hepatocytes Are Oval Cell Precursors

***BD Tarlow**†, W Naugler[#], L Wakefield[†], C Pelz[†], EM Wilson[‡], M Grompe[†]**

[*]Cell & Developmental Biology, [†]Pediatrics, [#]Gastrogenterology & Hepatology, Oregon Health Science University, Portland, OR; [‡]Yecuris, Inc., Tualatin, OR

Atypical ductal proliferation, or oval cell activation, is a hallmark of chronic liver injuries such as alcoholic cirrhosis, hepatitis B, and hepatitis C. Oval cells are thought to represent activated stem/progenitor cells that function as hepatocyte precursors. But the origin of oval cells is the subject of a long-term debate in the field. Recent reports indicate that adult mouse hepatocytes may contribute to the ductal reaction following forced gene expression or injury. Here, we asked whether mature human hepatocytes also give rise to oval cells in a mouse xenograft model. **Methods**: Normal human hepatocytes were isolated from surgical liver

resections or purchased from commercial sources. Hepatocytes were transplanted into the spleen of immune-compromised *Fah*$^{-/-}$ mice and allowed to repopulate. Highly-repopulated mice (25-75% humanization) were identified by human serum albumin levels for further study. Cell-type contributions at baseline and after injury were determined by immunohistochemical co-localization of human specific nuclear antigen with cell fate markers and species-specific transcriptional profiling (RT-PCR and RNAseq). Oval cell injury was induced with the compound DDC (3,5-diethoxycarbonyl-1,4-dihydrocollidine, 0.1% food). **Results**: At baseline, the only human hepatocytes (HNF4a+ and FAH+) but not human biliary (KRT19-, SOX9-) or human blood/endothelial cells (CD45- CD31-) were found to engraft in the *Fah-/-* mouse liver. Next, humanized chimeric mice were given an oval cell activation injury for 6-to-8 weeks. In 5 of 12 injured chimeric animals, we identified robust evidence of human hepatocyte-derived oval cells. Human cells with ovoid nuclei, scant cytoplasm, and an elongated ductal morphology were observed in the portal region immediately adjacent to proliferative murine biliary cells. These human cells showed decreased expression of hepatocyte marker FAH and increased expression of biliary markers hEpCAM, hSOX9, and hKRT19. **Conclusion**: Experimental oval cell injury induces a ductal/biliary phenotype in human hepatocytes. Mature human hepatocytes can contribute to atypical ductal reactions *in vivo.*

Conflict of interest: M Grompe and E Wilson have a significant financial interest in Yecuris, Inc.

184

Chemotherapy That Targets Regulatory T Cells, Given with Immunotherapy, Prevents Tumor Growth in a Murine Model of Claudin-Low Breast Cancer

NA Taylor, **MD Iglesia, BG Vincent, KP McKinnon, ML West, JE Burgents, CM Perou, JS Serody**

University of North Carolina at Chapel Hill, Chapel Hill, NC

Breast cancer is the leading cancer diagnosis and second leading cause of cancer death in women in the United States. Using gene expression array analyses from women with breast cancer, we distinguished 6 intrinsic subtypes of breast cancer: Luminal A, Luminal B, Normal-like, HER2, Basal-like, and Claudin-low. Basal-like and claudin-low subtypes possess the worst prognosis due to lack of targeted therapy, higher stage at diagnosis, and predilection for early metastasis. The claudin-low subtype is further separated from the basal-like subtype due to increased expression of proliferation markers and immune genes characteristic of tumor infiltration by immune cells. Although claudin-low tumors demonstrate a large immune infiltrate, it is unclear whether these cells are providing an effective anti-tumor response or promoting tumor growth. Using genetically modified murine tumor models of the different intrinsic subtypes developed by our group on the same p53$^{-/-}$ BALB/c background, we characterized differences in the immune response among the different subtypes. We found that claudin-low tumors (T11 model) recruited larger numbers of regulatory T cells (T_{regs}) to the tumor site, compared with luminal tumors (2250 model). Surprisingly, this accounted for up to 68% of all CD4^{+} T cells recruited to the T11 tumor site (range: 9-68%). Further, increased numbers of T_{regs} correlated with decreased numbers of CD8^{+} effector T cells and CD19^{+} B cells. Previously, our group found that cytotoxic chemotherapy that does not deplete T_{regs} had little impact on the growth of T11 tumors, with all mice dying by day 30 post-tumor implantation (PTI). Selective or specific depletion of T_{regs} by low-dose cyclophosphamide (Cy) or use of FoxP3-DTR mice, respectively, delayed tumor growth (median survival: 35 days); however, all mice succumbed to tumor by day 40. The combination of Cy with anti-PD1 and anti-CTLA4 antibodies improved median survival to 46 days PTI, compared to 23 days for untreated mice ($p < 0.0001$). Furthermore, 12% (n = 3/25) of mice treated with combination therapy failed to develop tumors. Administration of anti-PD1 and/or anti-CTLA4 antibody had no effect on survival. These data indicate that the optimal approach to treating claudin-low breast cancer clinically may be a combination of cytotoxic chemotherapy that can target T_{regs}, with immunotherapy that can enhance the local adaptive immune response.

185

Chimeric-Antigen Receptor Engineered Human T Lymphocytes from Induced Pluripotent Stem Cells for Cancer Therapy

Maria Themeli, **Christopher C. Kloss, Giovanni Ciriello, Michel Sadelain**

Memorial Sloan-Kettering Cancer Center, New York, NY

Progress in adoptive T cell therapy for cancer and infectious disease is hampered by the lack of readily available antigen-specific human T lymphocytes. Pluripotent stem cells could provide an unlimited source of T lymphocytes. We hypothesized that genetic engineering of induced pluripotent stem cells (iPSC) with Chimeric Antigen Receptors (CARs) would be an efficient strategy to concomitantly harness the unlimited availability of iPSC and to generate phenotypically defined, functional and expandable T cells that are genetically targeted to a tumor antigen of interest. Thus, we generated iPSCs from peripheral blood lymphocytes (T-iPSC) and we genetically engineered them to express 1928z, a second-generation CAR specific to the B-cell lineage antigen CD19. Stably transduced 1928z-T-iPS lines were then differentiated in vitro towards the T lymphoid lineage. By day 30 of differentiation around 80% of the cells were CD3+TCR$\alpha\beta$+ and expressed the 1928z CAR on the surface (1928z-TiPSC-T). 1928z-TiPCS-T cells rapidly responded to antigen encounter by forming clusters, increased the expression of activation markers and secreted type 1 cytokines. We further characterized the T-iPSC-derived T cells by performing a gene expression array and comparing their profile to that of known lymphoid cell subsets. Interestingly, 1928z-TiPSC-T cells showed the highest correlation with the expression profile of cultured peripheral blood $\gamma\delta$-T cells. We were able to expand 1928z-TiPSC-T cells around 1000-fold after 3 weekly stimulations. Expanded 1928z-T-iPSC-T cells displayed high antigen-specific cytotoxic activity in in vitro 51Cr release assays. To investigate the anti-tumor activity of 1928z-T-iPSC-T cells in vivo, NOD-SCID IL2Rγcnull mice were inoculated with the CD19+ Raji human Burkitt lymphoma cell line that expresses a fluorescent-luciferase fusion protein. Treatment with 1928z-TiPSC-T cells delayed tumor progression to an extent similar to that induced by peripheral blood 1928z-$\gamma\delta$ cells, and resulted in a significant survival advantage compared to untreated controls.

Poster Abstracts

In summary, the combination of iPSC and CAR technologies we describe here offers a tantalizing new source of "off the shelf" T cells of predetermined antigen specificity and an excellent platform for safely introducing additional genetic modifications to augment the potency and histocompatibility of immune effectors.

186

Osteoclast-Mediated Activation of Devitalized Engineered Cartilage to Generate Osteoinductive Grafts

A Todorov, **C Scotti, A Barbero, I Martin, A Papadimitropoulos**
Department of Biomedicine, University Hospital Basel, Switzerland

Background: Adult bone marrow stem cells can form hypertrophic cartilage (HC) in vitro which has the capacity to efficiently remodel into bone in vivo through the process of endochondral ossification. However, clinical application of this approach, e.g. in large segmental bone defects, faces challenges such as standardization, availability and immunogenicity. **Aim:** A strategy of high clinical and commercial interest is to create a devitalized HC with the same potential, which can be stored and used off-the-shelf. Yet thus far devitalized HC has not been able to efficiently remodel and vascularize in vivo. This study investigated whether devitalized HC can be activated by peripheral blood-derived osteoclast progenitors prior to implantation to induce ectopic bone tissue formation. **Methods:** HC were devitalized by successive cycles of freeze/thaw and either co-cultured with freshly isolated and sorted CD14+ monocytes from human peripheral blood, or cultured alone, in the presence of osteoclastogenic factors (MCSF; RANK-ligand) for one day. Samples were implanted subcutaneously in nude mice for up to 12 weeks. In parallel, samples were kept in culture for up to 23 days. **Results:** Co-cultured constructs remodeled into frank bone tissue and displayed higher morphological organization, greater mineralization and less remnants of cartilagineous tissue as compared to cell-free devitalized HC in vivo. Formation of osteoclasts and digestion of the matrix were observed during prolonged in vitro co-culture. **Conclusion:** The addition of osteoclastic cells on devitalized HC likely primed the onset of the remodeling process, which is a critical trigger of endochondral ossification. These findings could make it feasible to produce HC on a large scale as an off-the-shelf, devitalized product to be activated/stimulated using easily available autologous cells.

187

Alteration of Intestinal Microbiota in Response to Induced Immune System

Hao Tran, **Benoit Chassaing, Andrew Gewirtz**
Center for Inflammation, Immunity, and Infection, Georgia State University, Atlanta, GA

The intestinal microbiota is composed of a myriad of microorganisms that reside in our digestive tract and play important, beneficial roles for the host. They are central in carbohydrate degradation, nutrient absorption, vitamin synthesis, and competition against pathogenic bacteria that try to colonize our intestinal tract. The composition of the microbiota, once stabilized, is thought to remain stable throughout the life of the host; however, drastic changes in diet, physical activity, or environment can induce lasting compositional changes, as during pregnancy and use of broad-spectrum antibiotics. The importance of the microbiota is highlighted by the number of diseases associated with alterations in its composition, such as inflammatory bowel diseases, colitis, obesity, and many types of cancers. Re-stabilization and "normalization" of the gut microbiota would prove to be an effective tool in addition to the standard means of treatment for these illnesses. Our group hypothesizes that such an alteration can be achieved through an induced adaptive immune response against the established microbiota, since it is now well establish that cooperation between the innate and adaptive immune systems of the host maintains homeostasis of the microbiota. In our experimentation, we used weekly intraperitoneal injections of flagellin to induce the adaptive immune response in three groups of mice of varying immune ability. The mice used were ten RAG-1 knockout mice that lacked an innate immune response, nine TLR5/NLRC4 double knockout mice that lack an adaptive immune response, and ten wild type mice. The body weights of these mice were recorded, and fecal and blood samples were collected. From these samples, we can measure immunoglobulin and cytokine response to flagellin using ELISA and qPCR techniques to confirm the establishment of a strong adaptive immune response. In addition, bacterial DNA extracted from fecal samples and sequenced through Illumina technology can be used to analyze microbiota composition. Potentially, these weekly injections trigger the mouse immune system to mount a response against subgroup of bacterial population, especially against bacterium expressing flagellin, leading to a significant change in the composition of the intestinal microbiota.

188

Novel Biotribological Model of Cartilage Damage: Extracellular Matrix Changes

RL Trevino, **CA Pacione, S Chubinskaya, AM Malfait, MA Wimmer**
Rush University Medical Center, Chicago, IL

Background: Current *in vitro* models of post-traumatic osteoarthritis investigate disease progression after traumatic impaction through culture studies. However, these models do not apply additional damage wear as *in vivo*. We report a novel test sequence where surface damage is induced by articulation against polyethylene, followed by articulation against cartilage or cobalt chromium.

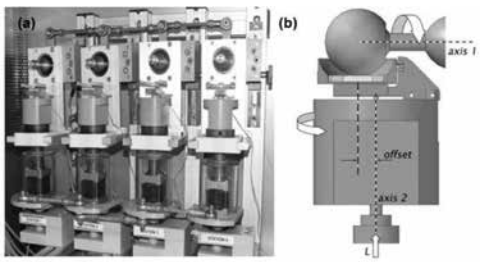


Figure 1: (a) Joint motion simulator, housed in incubator. (b) Model of contact between the ball and explant.

Methods: Bovine cartilage explants were randomized into damaged or healthy and tested under three conditions: against-cobalt chromium, against-cartilage, free-swelling. Tribological testing used a joint motion simulator at physiological conditions (Fig.1), utilizing a moving contact

point that preserves biphasic lubrication. Samples were loaded to 40N (~2MPa) and articulated against assigned countersurfaces. Testing was conducted for three hours per day for five days with damage induced by articulating explants against a polyethylene ball for the first two days. Tissue was examined for cell viability and for qualitative matrix assessment by histology for proteoglycan and collagen content. Release of proteoglycans was measured in test media. **Results**: Cell viability decreased in damage samples compared to their respective healthy controls. While damage produced significantly more cumulative proteoglycan release over the entire tribological test period compared to the respective healthy ($p<0.05$), there were no significant differences in proteoglycan release during test periods when polyethylene articulation did not occur. Histologically, damage displayed increased birefringence with Picrosirius Red, indicating more disruption of surface collagen versus healthy. Articulated samples (both healthy and damage), though, exhibited increased Safranin-O staining as compared to free-swelling, representing increased proteoglycan levels. **Discussion**: This study addresses the mechanobiological response of healthy and damaged cartilage due to articulating motion and load against various contact surfaces. The results suggest that understanding matrix wear is a complex process, especially when accounting for biological activity. Future studies shall include a more extensive analysis of the biosynthetic response and soluble markers of proteoglycan and collagen wear damage and metabolism.

189

Dysregulation and Recurrent Mutation of miRNA-142 in *de novo* AML

M.C. Trissal*, **J. Hundal†, S. McGrath†, V. Magrini†, E. Mardis†, T.J. Ley‡, D.C. Link‡**

**MD-PhD Program, Washington University in St. Louis, St. Louis, MO; †The Genome Institute at Washington University, St. Louis, MO; ‡Dept. of Internal Medicine Washington University, St. Louis, MO*

We recently reported whole genome or exome sequencing on 200 cases of *de novo* AML (The Cancer Genome Atlas, NEJM 2013). Recurring point mutations of only one microRNA (miR) gene, *MIR142*, were identified in 2% of patients. The miR "seed" sequence is critical in mediating the specificity of miR-mRNA target interaction; all *MIR142* point mutations localized to this critical seed region in miR-142-3p. Target prediction programs show loss of most predicted miR-142-3p targets upon mutation and no collectively shared mRNA targets between the mutants. Additionally, these mutants were unable to repress the miR-142-3p target, *RAC1*, *in vitro*. These data suggest these mutations likely lead to a complete loss of miR-142-3p function. Surprisingly, when we over-expressed *MIR142* mini-genes containing these miR-142-3p mutations, we observed decreased expression of the non-mutated miR-142-5p partner strand. To validate this *in vivo*, we sequenced the small RNA transcriptome of 28cases of *de novo* AML, including four cases harboring *MIR142* mutations. In general, miR-142-5p is expressed at higher levels than 3p (average ratio of miR-142-5p/3p: 1.8 ± 0.23). In the cases with *MIR142* mutations, a significantly decreased expression of miR-142-5p relative to 3p was observed (0.41± 0.13). This suggests that the mutations in the seed sequence of miR-142-3p affect both miR-142-3p mRNA targeting ability and result in decreased levels of functional miR-142-5p. Expression data revealed that miR-142-5p and 3p levels were increased 16± 3.6 and 30 ± 7.0 fold, respectively, in AML compared to control healthy donor marrow. We examined the effect of enforced expression of wild-type or mutant *MIR142* on hematopoiesis. Murine hematopoietic progenitors transduced with wild-type (WT) or mutant *MIR142* lentivirus and transplanted into recipients. Over-expression of WT miR-142 resulted in a drastic loss of repopulating activity. In contrast, cells transduced with mutant *MIR142* were able to generate multi-lineage engraftment for at least 7 months post transplant. This suggests miR-142 over-expression in AML may be compensatory to transformation and serve to inhibit leukemic cell growth. The loss-of-function of *MIR142* observed in AML may disrupt this negative feedback loop, thereby promoting AML expansion. Studies to characterize the miR-142 target repertoire within the hematopoietic system are currently underway and should provide insights on how disruption of miR-142 function may contribute to the pathogenesis of AML.

190

Acute Lymphoblastic Leukemia Cells Utilize Adipocyte-Derived Free Fatty Acids for Proliferation

J Tucci, **X Sheng, SD Mittelman**

Children's Hospital of Los Angeles, Los Angeles, CA; University of Southern California, Los Angeles, CA

Background: Obesity promotes the pathogenesis of many cancers, including acute lymphoblastic leukemia (ALL), the most common childhood malignancy. We have shown that ALL cells interact with adipocytes in vitro and in vivo, and that adipocytes protect ALL cells from chemotherapies. Since cancer cells require free fatty acids (FFA) for energy and as molecular building blocks, we hypothesize that ALL cells gain a survival advantage by stimulating adipocyte lipolysis and using adipocyte-derived FFAs. **Methods:** To test whether ALL cells stimulate adipocyte lipolysis, 3T3-L1 adipocytes were exposed to ALL-conditioned media for 72 hours, and glycerol release and adipocyte lipid content were measured. Adipocytes were pre-labeled with fluorescent FFA (BODIPY) and co-cultured with murine and ALL cells in a Transwell system. ALL uptake of adipocyte BODIPY-FFAs was analyzed via flow cytometry. To determine the fate of FFA in ALL cells, ALL cells were incubated with BODIPY-FFA and evaluated by thin layer chromatography (TLC), confocal and live cell microscopy. Real-time PCR was employed to analyze adipocyte-induced alterations in the transcription of key enzymes in the ALL de novo lipogenesis pathway. Finally, TOFA, an acetyl-CoA carboxylase 1 (ACC1) and steroyl-CoA desaturase 1 (SCD1) inhibitor, was used to block de novo lipogenesis in ALL cells in vitro and evaluate the ability of ALL cells to use adipocyte-derived FFAs for proliferation. **Results:** ALL-conditioned media stimulated 3T3-L1 lipolysis (191.0 vs. 165.5 mg/L glycerol, n=1), reducing adipocyte lipid content as quantified with Oil Red O by 16.8 ± 7.1% ($p = 0.047$). When murine or human ALL cells were co-cultured with BODIPY-labeled adipocytes, 98% of ALL cells accumulated the fluorescent label within 48 hours. TLC, live cell imaging and confocal microscopy demonstrated the majority of this label accumulated within ALL cell phospholipid membranes and triglyceride-laden lipid droplets. Adipocyte co-culture reduced expression of lipogenic enzymes such as fatty acid synthase (FAS),

Poster Abstracts

ACC1 and SCD1 in ALL cells. Finally, while TOFA inhibited ALL cell proliferation, this proliferation was partially rescued by adipocyte conditioned media. **Conclusion:** Therefore, we have demonstrated that ALL cells stimulate adipocyte lipolysis and use adipocyte-derived FFAs to supplement de novo lipogenesis and proliferation. This may contribute to the effect of obesity on ALL development and relapse.

191

High Fat Diet Induced Immune Selection of the Microbiome Optimizes Host Growth

***Vaibhav Upadhyay*[1,2], Kevin P. Keegan[3], Yuan Zhang[2], Tae-jin Kim[1,2], Kevin Muite[1,2], Folker Meyer[3], and Yang-Xin Fu[1,2]**
[1]Committee on Immunology, [2]Department of Pathology, [3]Mathematics and Computer Science Division and Institute for Genomics and Systems Biology Argonne National Laboratory

Background & Aims: It is now speculated that the immune system may contribute to the normal physiologic regulation of the microbiome. Studies have established both protective and risk variant alleles in the gene encoding lymphotoxin (LT), a component of colonic defense, and body size. Recent data suggests that LT- receptor (LTR) may be responsive to dietary intake but how the LT-pathway responds to diet and regulates the microbiome is not known. **Methods:** We generated *Ltbr*$^{-/-}$ germ free mice. These or colonized mice (*Lta*$^{-/-}$, *Ltbr*$^{-/-}$, and *Rorc*$^{-/-}$) were fed a normal or high-fat diet (HFD). We examined immune activity within the colon using flow cytometry and light microscopy. We interrogated regulation of the microbiome by shotgun sequencing cecal contents (>133 Gb of data). **Results:** HFD induced LT expression, and recruited B cells to the colon. HFD initiated the formation of lymphoid structures contingent on signals via LTR and the microbiota. Metagenomic sequencing revealed that both HFD and LTR were required to create a unique microbiome configuration. LTR repressed metabolism and DNA replication in colonizing species. Consistent with this, *Ltbr*$^{+/+}$ and *Ltbr*$^{-/-}$ germ free mice grew identically on HFD. Although essential in SPF hosts, LTR was dispensable for B cell homeostasis in axenic mice. **Conclusions:** Our data reveal that host immunity via the lymphotoxin pathway responds to HFD intake and effectively regulates the microbiome to secure nutrition for host growth but is not essential when microorganisms are absent.

192

Vaccination with PSA146-154 for Advanced Prostate Cancer: A 10 Year Follow-Up

Vivek Upadhyay**, David Peace*, Supriya Permabakam, Hui Xie
University of Illinois-Chicago, Chicago, IL

Background: Prostate cancer is the second leading cause of cancer-related death among men in the United States. Although localized disease may be definitively treated with prostatectomy or radiotherapy, advanced metastatic disease can only be managed transiently with androgen-deprivation therapy (ADT). Given the prevalence and lack of curative options for advanced disease, novel treatments warrant investigation. A UIC study in 2002 examined whether vaccination against PSA146-154 elicited specific T cell immunity in 28 patients with advanced disease. Ten years following immunization, this study examines the long-term effects of the vaccine. Individuals who demonstrated a DTH response against PSA146-154 peptide have improved survival compared to those individuals who did not yield a DTH response against the peptide. Likewise, IFN gamma and tetramer responses against the PSA peptide do not correlate with improved survival. RNA analysis of DTH responders reveals increased expression of genes involved in plasmacytoid dendritic cell function. Together, these findings suggest that vaccination against the PSA146-154 peptide may lead to improved survival in patients who effectively mount a DTH response. Furthermore, the RNA gene expression analysis suggest an antitumor role for plasmacytoid dendritic cells in prostate cancer.

193

White Matter Microstructure Abnormalities in the Fornix and Cingulum of Cigarette Smokers

***A.R. Verde**, V.W. Chanon, M. Ahn, E. Cerciello, H. Zhu, M.A. Styner, and C.A. Boettiger**
**University of North Carolina, Chapel Hill, NC*

Purpose*:* With cigarette addiction's one year quit success rate of only 5%, more effective therapies are greatly needed. A better understanding of the neurobiology of cigarette addiction may help to advance this effort. People with addictive disorders characteristically select smaller, sooner over larger, delayed rewards, and the neurobiology of such decision-making is beginning to come to light. For instance, recent studies in healthy people show that the use of episodic prospection during decision-making can reduce impulsive choices, an effect mediated by enhanced synchrony between medial temporal lobe regions implicated in episodic prospection and medial prefrontal areas implicated in decision-making. As such, we hypothesized that regular smokers would show structural abnormalities in the white-matter tracts through which these brain areas communicate: the cingulum, fornix, and uncinate. Previous studies of white-matter in smokers using diffusion tensor imaging (DTI) have yielded inconsistent results, and no prior studies report data from the fornix. Here, we used a novel DTI tractography analytical approach assessing diffusion properties along our tracts of interest to quantify differences in white matter microstructure integrity associated with smoking. In addition, we measured correlations between white matter integrity and measures of addiction severity, and cigarette consumption. **Methods:** We obtained diffusion weighted images (DWI) from nonsmokers (n=15) and smokers (n=10), ages 19-40. Images were processed and analyzed using the UNC-Utah NA-MIC Framework for DTI Fiber Tract Analysis, including rigorous quality control, a study specific atlas, tractography, and analysis along the tract of diffusion parameters (fractional anisotropy, radial diffusivity, axial diffusivity, mean diffusivity). **Results:** We found decreased white matter integrity in bilateral fornix crus of smokers relative to nonsmokers. Among smokers, white matter integrity in these tracts, as well as the anterior and body of bilateral superior cingulum positively correlated with measures of both cigarette consumption (including CO levels) and cigarette dependence. **Conclusions:** White matter microstructural integrity is decreased in smokers compared to non-smokers in the fornix crus. However, within smokers, those with increased addiction

severity have increased integrity in this same area of the fornix, as well as in the body and anterior of the cingulum. This alteration in white matter integrity could reflect consequences of cigarette consumption and/or addiction, but could also reflect pre-existing structural difference in the brains of smokers, which would represent possible risk-factors.

194

Interleukin-1 Beta Inhibits Hepatitis C Virus Infection

***Cesar A. Virgen*[1,2] and Francis V. Chisari[1]**

[1]Department of Immunology and Microbial Science, The Scripps Research Institute and [2]University of California San Diego, School of Medicine, La Jolla, CA

Hepatitis C virus (HCV) triggers a necroinflammatory liver disease that can progress to steatosis, cirrhosis, and hepatocellular carcinoma. With more than 170 million people chronically infected worldwide, HCV is a major public health concern. Aside from the effect of interferon alpha, little is known about the ability of circulating biomolecules to activate hepatocyte intrinsic cellular defenses that can regulate HCV infection. Therefore, we set out to test the impact of a panel of 15 recombinant cytokines and 9 hormones on HCV JFH-1 genotype 2a infection of the Huh-7 human hepatoma cell line. Surprisingly, we found that Interleukin-1 alpha (IL-1a) and Interleukin-1 beta (IL-1b) significantly inhibited HCV JFH-1 infection without affecting cell viability. Both cytokines are known to bind to the type 1 Interleukin-1 receptor (IL-1R1) and trigger a signaling cascade that leads to NF-κB activation. To our knowledge, this is the first study implicating the IL-1R1 signaling pathway in the control of HCV infection. IL-1b is the prototypical proinflammatory cytokine and several studies have shown that patients with chronic HCV infection have elevated serum IL-1b levels. Recently, it was reported that HCV induces inflammasome activation that leads to production of IL-1b in an immortalized macrophage cell line, and that Kupffer cells from chronic hepatitis C patients produce IL-1b. We hypothesize a novel antiviral role for IL-1b in the liver since our findings suggest that IL-1a and IL-1b trigger the control of the viral infection within Huh-7 cells. We are currently characterizing the stage(s) of the HCV life cycle that is/are inhibited by the IL-1R1 signaling pathway by using the HCV JFH-1 infectious clone and other systems. In addition, we are performing gene expression analysis to identify the direct mediators of this antiviral activity. Understanding how the Interleukin-1 receptor signaling pathway regulates HCV infection may lead to better understanding of the intrinsic cellular defense mechanisms involved in control of HCV infection.

195

Loss of *Nfkb1* Results in Accumulation of DNA Damage and Early Animal Aging

JS Wahlstrom**, GM Bernal*, CD Crawley*, KE Cahill*, P Pytel*, H Liang*, S Kang*, RR Weichselbaum*, and B Yamini

**The University of Chicago, Chicago, IL*

Background: DNA damage plays a critical role in the development of chronic disease and aging. NF-□B modulates the cellular response to DNA damage, and recently the NF-□B1/p50 subunit was identified as a central factor in the cytotoxic response to DNA damage. We followed a colony of *Nfkb1*$^{+/+}$, *Nfkb1*$^{+/-}$ and *Nfkb1*$^{-/-}$ littermate mice to determine whether an association exists between *Nfkb1* and aging. Although *Nfkb1*$^{-/-}$ mice appear similar to their littermates at a young age, they develop several observable age-related phenotypes earlier than wildtype counterparts. Also, *Nfkb1*$^{-/-}$ mice develop increased kyphosis and decreased cortical bone fraction compared to *Nfkb1*$^{+/+}$ mice. In addition, a significant increase in GFAP staining is noted in the brains of 12-month old *Nfkb1*$^{-/-}$ mice relative to *Nfkb1*$^{+/+}$. Most significantly, *Nfkb1*$^{-/-}$ animals have a decrease in overall lifespan compared to their littermates. *In vitro*, primary *Nfkb1*$^{-/-}$ MEFs decrease their population doubling rate compared to *Nfkb1*$^{+/+}$ over serial passages due to increased senescence and reduced proliferation. Examination of γH2AX staining demonstrates an increase in foci both in cultured MEFs and in brains of *Nfkb1*$^{-/-}$ animals compared to *Nfkb1*$^{+/+}$. Finally, although overall NF-□B binding increases in the brain of old wildtype animals compared to young, a substantial decrease in p50 DNA binding is seen. Collectively, these findings indicate that loss of *Nfkb1* leads to early aging associated with increased DNA damage accumulation and that wildtype animals lose p50 DNA binding with age. Together, these data suggest NF-□B1/p50 plays a role in the physiological aging process and provide a signaling pathway that may be targeted for therapeutic amelioration of age-related disease processes.

196

A Literature Review of Mentorship in Academic Medicine: Guidelines to Improve Training Satisfaction Through Enhancing Mentorship Opportunities

***Xin Wang*[1], Enoch Ng[1], and Ju-Yoon Yoon[2]**

[1]MD/PhD Program, University of Toronto, Toronto, ON, Canada; [2]MD/PhD Program, University of Manitoba, Winnipeg, MB, Canada

Background: The Clinician Investigator Trainee Association of Canada/ Association des cliniciens-chercheurs en formation du Canada (CITAC/ACCFC) actively promotes and supports clinician investigator trainees in Canada. Part of this mandate involves the curation of trainee demographics and survey data to improve training success and satisfaction. CITAC recently published the first survey to assess factors that contribute to satisfaction. One of the key findings of this survey is that increased level of mentorship was strongly correlated with overall satisfaction. However, despite over 98% of survey respondents reported mentorship as important to their success, more than 60% expressed some level of dissatisfaction with the level of mentorship received. To address this discrepancy, this literature review assesses mentorship in

academic settings with a focus on clinician investigator trainees with the aim of developing strategies to increase the level of mentorship among trainees. **Methodology:** We identified 198 articles from OVID in addition to manual curation based on the search term 'Mentorship' AND 'Education, Medical, and Research'. Two of the authors independently reviewed the titles and abstracts and narrowed down to 75 articles based on relevance to mentorship in academic medicine. Consensus of decision resulted in the selection of 20 articles for complete review. **Results:** Despite the importance of mentorship in clinician investigator trainee satisfaction, it is surprising that there is a paucity of studies investigating the role of mentorship and the impact to trainee success and retention. Several themes of mentorship have been identified and discussed in this review including: 1) Importance and benefits of mentorship, 2) Qualities of a good mentor, 3) How to establish a positive mentor-mentee relationship, and 4) Programs to facilitate mentorship in academic medicine. Recommendations for improvement or change in clinician investigator trainee programs are discussed herein. Having identified the importance of mentorship in academic medicine, it is crucial to implement programs to better train mentors and provide opportunities for sustained mentor-mentee relationships.

197

Targeting NMDAR-Hypofunction Reverses Behavioral Deficits in a Mouse Model of Frontotemporal Dementia

***BA Warmus*[*], DR Sekar[*], GD Schellenberg[†], LL McMahon[*], ED Roberson[*]**

[*]University of Alabama at Birmingham, Birmingham, AL; [†]University of Pennsylvania School of Medicine, Philadelphia, PA

Frontotemporal dementia (FTD) is a rapidly progressive and lethal disease with no disease-modifying treatments. Tau mutations cause FTD, but the underlying neurobiology has not been well defined. Here, we address this question using a new mouse model (hTauV337M) with selective vulnerability of the insula/ventral striatum and abnormalities including repetitive behavior, both of which are features of FTD. We found that mutant tau depletes PSD-95 and impairs synaptic localization of AMPARs and NMDARs selectively in brain regions affected in human FTD. Patch-clamp recordings from ventral striatum indicated AMPAR- and NMDAR-hypofunction. Targeting NMDAR-hypofunction with the FDA-approved D-cycloserine, an NMDAR co-agonist, reversed behavioral abnormalities. These results indicate that mutant tau impairs NMDAR trafficking, causing NMDAR-hypofunction in vulnerable brain regions, and that this process can be therapeutically targeted. These findings have important treatment implications, including the possibility of repurposing D-cycloserine for treating FTD.

198

DNA Repair Deficiency and Sensitivity to PARP Inhibition in HPV-Positive Head and Neck Cancer

***Alice N. Weaver,* Tiffiny Cooper, Hoa Q. Trummel, James A. Bonner, Eddy S. Yang**

University of Alabama at Birmingham, Birmingham, AL

Background*:* Incidence of human papillomavirus (HPV)-positive head and neck cancer (HNC) has risen sharply in recent years. These tumors significantly differ from their HPV-negative counterparts, including expression of HPV oncoproteins E6/E7 and increased response to both radio- and chemotherapy. Due to their favorable response rate, it is of great importance to identify de-intensified anticancer therapy to avoid excessive treatment-related toxicities in patients. Poly (ADP-ribose) polymerase (PARP) inhibitors are agents that selectively target DNA repair-deficient cancers with only mild side effects. As HPV E6 and E7 are known to induce persistent DNA damage and chromosomal instability, we hypothesized that HPV-positive HNC has reduced DNA repair capability sufficient to be rendered sensitive to PARP inhibition. **Methods***:* DNA damage was assessed by neutral comet assay and immunihistochemistry for markers of homologous recombination repair (HR) and non-homologous end joining (NHEJ). Response to PARP inhibition was examined *in vitro* with colony formation assays and *in vivo* with heterotopic explants, including a patient-derived HPV-positive xenograft. **Results:** HPV-positive HNC demonstrated elevated baseline levels of DNA damage in addition to abnormal DNA damage resolution following radiation. Decreased markers for HR and NHEJ indicate possible defects in both these pathways. These findings coincided with a significant response to PARP inhibition in *in vitro* and *in vivo* models. **Conclusions:** HPV-positive HNC harbors DNA repair defects in both HR and NHEJ mediated by an as-yet unknown mechanism, correlating with sensitivity to PARP inhibition. These studies identify PARP inhibitors as a potential agent for de-escalated anticancer therapy in HPV-positive HNC patients.

199

Neuroprotective Effects of Dimethylfumarate Against MPTP Mouse Model of Parkinson's Disease

***Mason Webb*[1], Lichuan Yang[2], Navneet Ammal Kaidery[2], Shaista Tarannum[2], Natalya Smirnova[4], Minyoung Jin[2], Swati Wakade[2], Rajiv Ratan[4], Irina Gazaryan[4], Bobby Thomas[2,3]**

[1]MD PhD program Medical College of Georgia, [2]Department, Pharmacology, and Toxicology, [3]Neurology, Medical College of Georgia, Georgia Regents University, Augusta; [4]Department of Neurology, Burke Cornell Medical Centre, White Plains, NY

Background: There is substantial evidence from patients and from animal models that oxidative damage plays a major role in the pathogenesis of the dopaminergic neuron degeneration in Parkinson's disease (PD). Preventing oxidative stress either by providing exogenous antioxidants or by enhancing endogenous antioxidative capacity has been intensely investigated for PD therapies. The latter includes the activation of nuclear-factor-E2-related factor 2 (Nrf2)/antioxidant response element (ARE) signaling pathway which regulates the expression of a battery of genes encoding anti-oxidative and cytoprotective genes.

Poster Abstracts

Fumaric acid ester dimethylfumarate have been found to exert neuroprotective effects by activating the Nrf2/ARE signaling pathway. We investigated effects of dimethylfumarate to activate Neh2-luciferase reporter *in vitro*, ability to activate Nrf2/ARE signaling in mouse embryonic fibroblasts from wild type and Nrf2 knockout mice, and its ability to block 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine toxicity in mice. We found that dimethylfumarate activates Nrf2 pathway using a Neh2-luciferase reporter, as assessment of mRNA and proteins levels showed upregulation of several cytoprotective and antioxidative genes in wild type mouse embryonic fibroblasts but not in Nrf2 null mouse embryonic fibroblasts *in vitro*. Intraperitoneal administration of dimethylfumarate at (10, 20, and 50mg/kg) dose dependently protected against acute MPTP (10mg/kg four times every 2 hour)-induced nigrostriatal dopaminergic neurotoxicity assessed by stereological cell counts of total and tyrosine hydroxylase positive neurons of substantia nigra pars compacta and striatal levels of catecholamines employing HPLC electrochemistry. Our results indicate that dimethylfumarate protects against nigrostriatal dopaminergic neurotoxicity by virtue of its ability to activate neuroprotective Nrf2/ARE and is a promising drug for PD.

200

Conotruncal Heart Defects and Genetic Variants in Folate, Homocysteine and Transsulfuration Pathways

DM Webber, **SL MacLeod, SW Erickson, MA Cleves, X Tang, J Li, M Li, TG Nick, CA Hobbs**

University of Arkansas for Medical Sciences, Arkansas Children's Hospital Research Institute, Little Rock, AR

Background: Conotruncal heart defects (CTDs) are among the most severe birth defects worldwide and result in significant morbidity and mortality. Folate-dependent methionine and homocystine metabolism is thought to play a role in such defects. To identify genes associated with CTD risk, we conducted a case-control study of single nucleotide polymorphisms (SNPs) in genes within the folate, homocysteine and transulfuration candidate pathways. The identified putative risk genes were then sequenced among affected cases. **Methods:** Participants were enrolled in the National Birth Defects Prevention Study between 1997 and 2007. DNA samples from 616 case-parental triads affected by CTDs and 1,645 control-parental triads were genotyped using an Illumina Golden Gate custom SNP panel. A hybrid, log-linear approach was used to identify maternal and fetal SNPs associated with CTDs. We then applied targeted sequencing to a subset of 379 cases to detect functional variants within identified risk loci. **Results:** Among 921 SNPs, 17 maternal and 17 fetal SNPs had a Bayesian false-discovery probability (BFDP) of <0.8. Ten of the 17 maternal SNPs and 2 of the 17 fetal SNPs were found within the glutamate-cysteine ligase, catalytic subunit (GCLC) gene. Fetal SNPs with the lowest BFDP (rs2612101, rs2847607, rs2847326, rs2847324) were found within the thymidylate synthetase (TYMS) gene. *In-silico* analysis identified likely functional variants within sequenced genes. **Conclusions:** These results support previous studies suggesting that maternal and fetal SNPs within folate, homocysteine, and transsulfuration pathways are associated with CTD risk.

201

Clearance of Human Metapneumovirus Infection Occurs Independently of Natural Killer Cells

SC Wen*, **K Boyd****, **SJ Tollefson****, **M Johnson****, **JV Williams****†

Vanderbilt University School of Medicine, Departments of Pediatrics† and Pathology, Microbiology, and Immunology, Nashville, TN*

Human metapneumovirus (HMPV), a paramyxovirus discovered in 2001, is a major cause of acute respiratory tract disease in children, the elderly, and immunocompromised individuals worldwide. There is currently no available vaccine for HMPV, and the mechanisms of protective immunity to this single-stranded RNA virus are not clear. Natural Killer (NK) cells are lymphocytes of the innate immune system that generally respond to viral infections by releasing cytokines and by direct cytotoxicity to infected cells. However, in the lung environment, immune responses must be balanced so that pathogens are cleared, but immunopathology resulting in airway occlusion and impaired gas exchange does not occur. There are still major gaps in our understanding of how NK cell function is regulated in the lungs – while NK cells have protective functions against many infections, they have also been shown to worsen disease by increasing lung inflammation during certain respiratory infections. Furthermore, no published studies have directly assessed NK cells in host protection during HMPV infection. To determine the role of NK cells during the host immune response to HMPV, we infected C57BL/6 mice with the virus and found that infected mice had higher numbers of NK cells recruited to their lungs as compared to mock-treated controls. NK cell recruitment was evident by day 1 post-infection and reached a peak on day 3. These NK cells were activated, as determined by flow cytometry after staining for IFN and CD107a (a marker for degranulation). However, although HMPV-infected mice had higher numbers of activated and functional NK cells in their lungs as compared to mock-treated mice, this increase did not correlate with host control of viral infection, as NK-depleted mice were able to clear virus as quickly as control mice. There were also only modest differences between NK-depleted and control mice in lung histopathology and cytokine production. Furthermore, there was little difference between NK-depleted and control mice in terms of CD8+ and CD4+ T cell number and function during the adaptive immune response. These observations indicate that in contrast to the well-established role of NK cells in many other viral infections, their contribution to the host control of HMPV is inessential.

202

Catalytic Mechanism of the Usher in Pilus Biogenesis by Uropathogenic *Escherichia coli*

GT Werneburg*†, **DG Thanassi†**

**Medical Scientist Training Program, Stony Brook University, Stony Brook, NY; †Department of Molecular Genetics & Microbiology, Stony Brook University, Stony Brook, NY*

The chaperone/usher (CU) pathway is a conserved secretion system employed by many species of Gram-negative bacteria to construct virulence-associated surface structures known as pili or fimbriae. Our model system, type 1 pili, facilitates colonization of the bladder by uropathogenic *Escherichia coli*. The CU pilus biogenesis pathway requires both a periplasmic chaperone

protein and an integral outer membrane protein, the usher. The chaperone facilitates proper folding of pilus subunits in the periplasm and prevents premature interactions. The usher catalyzes the exchange of chaperone-subunit for subunit-subunit interactions, and promotes ordered polymerization and secretion of the pilus fiber. The usher has 5 domains: a periplasmic N-terminal domain (N), a transmembrane beta-barrel domain that is gated by an internal plug domain, and two periplasmic C-terminal domains (C1 and C2). Chaperone-subunit complexes first bind to the N domain of the usher and then move to the C domains as the plug is expelled and the pilus subunits are inserted into the barrel channel. To understand the mechanism of the usher (FimD) in catalyzing the assembly and secretion of pili, we are monitoring interactions of chaperone-subunit (FimC-FimH) complexes with usher domains during pilus assembly using fluorescence-based approaches. We calculated an overall affinity for FimC-FimH binding to the full length FimD usher that is in agreement with previous surface plasmon resonance measurements. We then combined our approach together with domain deletion mutants of FimD to reveal the contributions of individual usher domains to chaperone-subunit binding and gain insight into the sequential order of chaperone-subunit-usher interactions. Our data suggest that the C domains of the usher contribute substantially to the usher's affinity for chaperone-subunit complexes. However, in the absence of the usher's N domain, no binding was detected. These findings support a model wherein the C domains are only accessible once the chaperone-subunit complex binds the usher's N domain, and the N to C domain handoff is driven by differential affinity. We also show that in the context of the full-length usher, the plug domain does not contribute directly to FimD's affinity for chaperone-subunit complexes, but likely "masks" FimD's C domains prior to usher activation. Based on these findings, we are currently developing and implementing additional fluorescence and biochemical assays to further our understanding of the mechanism of the usher in the assembly and secretion of pili.

203

Exploring the Role of *Streptococcus pyogenes* Bacterial Communication in Colonization, Carriage and Virulence

***R Wilkening*[*], MJ Federle[†]**

[*]Department of Microbiology and Immunology, University of Illinois at Chicago, Chicago, Il; [†]Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL

Streptococcus pyogenes is a major cause of human disease that results in 600 million infections/year. Though the majority of infections result in a simple pharyngitis, *S. pyogenes* can also result in invasive diseases such as necrotizing fasciitis and numerous non-suppurative sequalae such as scarlet fever and rheumatic heart disease. It has been shown that the broad array of disease caused by *S. pyogenes* is in part due to its ability to modulate the human immune system, specifically the ability to limit an effective immune response. Bacterial cell-to-cell communication, also known as quorum sensing (QS), provides a mechanism by which bacteria coordinate gene expression across a microbial population to behave as a group. Furthermore, QS has been shown to modulate virulence in pathogens such as *Staph aureus* and *Escherichia coli*. With this knowledge in mind, our lab has identified a novel quorum-sensing pathway in *S. pyogenes*. The pathway consists of two transcriptional regulators, one activator (Rgg2) and one repressor (Rgg3) that are modulated by expression of a pair of short hydrophobic peptides (Shps). These peptides, produced intra-cellularly, are exported, processed to an active form (termed pheromones), and are re-imported into the cell. Once a threshold concentration is reached, the Shps bind their cognate Rggs and the system is activated by disruption of Rgg3 repression and the potentiation of Rgg2 activation. Activation of this system leads to expression of numerous downstream operons, the majority of which are poorly understood at this time. This project seeks to elucidate the roll of the Rgg2/3 quorum sensing pathway in modulating host-pathogen interaction during colonization, carriage and virulence states. We will explore these interactions using both *in vivo* and *in vitro* models to asses differential cytokine expression, cellular cytotoxicity, and immunogenicity of *S. pyogenes* with and without activation of the Rgg2/3 system. A more complete understanding of the importance of this system during disease states would allow the identification of new drugable targets, using a 'quorum quenching' approach to silence these pathways.

204

Elucidating Mechanisms of pH Sensing in the Proprotein Convertases

***DM Williamson,* J Elferich, U Shinde**

Department of Biochemistry and Molecular Biology, Oregon Health & Science University, Portland, OR

The Proprotein Convertases (PCs) are a family of serine endoproteases that are structurally and biochemically related to prokaryotic subtilisins and yeast kexins. The PCs are responsible for the conversion of a wide range of physiologically important proproteins to their active forms, including processing of proopiomelanocortin (POMC), proinsulin, and growth factor receptors, and thus play central roles in human development, heath and disease. We have previously demonstrated that the PCs are synthesized as inactive precursors that are folded and chaperoned through the secretory pathway by their propeptides, which function as intramolecular chaperones (IMCs). Additionally, we identified a conserved histidine residue in the IMC that functions as a pH sensor in furin, the canonical PC, to regulate its pH-dependent activation in the TGN. While this histidine is conserved across all PCs, each protease exhibits unique pH-optima for activation that corresponds with its organellar compartment of action. We have used furin, which is activated in the TGN, and its paralogue PC1/3, which is activated in mature secretory granules, as test cases to elucidate the unique determinants of pH-dependent activation of these proteases, and gain a better biophysical understanding of how IMCs drive activation of the PCs. We hypothesized that the IMCs of the PCs contain all of the necessary information to dictate pH- and organelle-specific activation of their cognate proteases, and thus subtle differences in the sequence and structure of the IMCs are responsible for their differential activation profiles. Our results suggest that the IMCs of furin and PC1/3 are sufficient to confer organelle-sensing on folding and activation of their cognate proteases, and that swapping IMCs can reassign pH-dependent activation in an

Poster Abstracts

IMC-dictated manner, yet we do not yet fully understand how these highly similar propeptides have such disparate effects on activation. Here, we present further biochemical, biophysical, and *in vivo* characterization of the IMC of PC1/3 that suggest subtle differences at the sequence level can yield significant differences on the role of IMCs as regulators of trafficking and activation the of PCs.

205

β-arrestin2 Contributes to Thoracic Aortic Aneurysm Formation in a Murine Model of Marfan Syndrome

***JW Wisler*[*], EM Harris[*], L Mao[*], MC Raisch[*], J Kim[*], HA Rockman[*], RJ Lefkowitz[*†]**

[*]Department of Medicine, Duke University Medical Center, Durham, NC; [†]Howard Hughes Medical Institute, Duke University Medical Center, Durham, NC

Thoracic aortic aneurysm (TAA) formation is the leading cause of morbidity and mortality in Marfan Syndrome. In addition to aberrant transforming growth factor-β signaling, angiotensin II type 1a receptor ($AT_{1A}R$)-mediated signaling including activation of mitogen-activated protein kinases (MAPK) contributes to TAA development in Marfan Syndrome. Given that β-arrestin2 (βarr2) is known to mediate $AT_{1A}R$-dependent MAPK activation as well as angiotensin II (ang II)-mediated abdominal aortic aneurysm formation, we hypothesized that βarr2 may contribute to TAA formation in Marfan Syndrome. Here we report that when *Fbn1*$^{C1039G/+}$ mice, a murine model of TAA formation in Marfan Syndrome, are crossed with βarr2$^{-/-}$ mice (*Fbn1*$^{C1039G/+}$/βarr2$^{-/-}$) a significant delay in TAA growth is observed compared to *Fbn1*$^{C1039G/+}$ animals, particularly at early time points. In addition, we observed significantly reduced mRNA and protein expression of multiple pro-fibrotic genes/proteins known to be involved in TAA formation in Marfan Syndrome in the aortic tissue of *Fbn1*$^{C1039G/+}$/βarr2$^{-/-}$ mice relative to *Fbn1*$^{C1039G/+}$ mice. These pro-fibrotic genes include matrix metalloproteinase (MMP) 2 and 9 as well as monocyte chemotactic protein (MCP)-1. In addition, Extracellular-regulated kinase 1/2 (ERK 1/2) phosphorylation is decreased in the aortas of *Fbn1*$^{C1039G/+}$/βarr2$^{-/-}$ mice compared to *Fbn1*$^{C1039G/+}$ animals. Stimulation of primary aortic vascular smooth muscle cells with ang II or the β-arrestin-biased agonist TRV120023 significantly increases mRNA and protein expression of several pro-fibrotic genes including MMP2, MMP9, and MCP-1. Moreover, inhibition of ERK 1/2 and blockade of the epidermal growth factor receptor inhibit ang II and TRV120023-dependent pro-fibrotic gene and protein expression whereas blockade of the transforming growth factor-β receptor has no effect on gene expression. Finally, small interfering RNA targeting βarr2 inhibits ang II or TRV120023-stimulated pro-fibrotic gene and protein expression. These results suggest that βarr2 contributes to TAA formation in Marfan Syndrome by mediating MAPK activation and subsequent expression of pro-fibrotic genes and proteins downstream of the $AT_{1A}R$. This represents a unique pathogenic signaling pathway in TAA formation in Marfan Syndrome and identifies βarr2 as a potential novel therapeutic target for inhibition.

206

Technology-Facilitated Depression Care Management Among Predominantly Latino Diabetes Patients within a Public Safety Net Care System: Provider Perspective

***Brian Wu*[1], Paul Di Capua[2], Shinyi Wu[3]**

[1]USC Keck School of Medicine, [2]PDC: UCLA David Geffen School of Medicine, Division of General Internal Medicine, [3]USC Epstein Department of Industrial and Systems Engineering

Background: Health disparities continue to exist in Latino diabetic patients despite evidence for improved care using primary care collaborative teams. Communication, economics, time, and motivation hinder both patients and providers from high quality care, but evidence-based practices can lower these barriers. However, implementation of these practices can be difficult, and provider adoption is critical for their success. **Purpose:** The Diabetes-Depression Care-management Adoption Trial (DCAT) aims to compare approaches to accelerating the adoption of collaborative team depression care and subsequently to optimize depression screening, treatment, follow-up, outcomes, and cost savings to reduce health disparities. Healthcare providers in each arm participated in a mixed-methods evaluation to assess changes in barriers to providing recommended care, the success of the information system implementation, and the role of cultural and organizational characteristics. **Methods:** 1406 low-income, predominately Hispanic/Latino patients with diabetes enrolled into the DCAT study and were split into three-groups that compared usual care, usual care with a collaborative care team support model, and a technology-facilitated depression care screening and monitoring tailored to patient conditions and preferences. Health professionals from all enrolled clinics participated in the surveys and interviews to provide feedback on the intervention. 37 total interviews were conducted: 4 social workers, 13 clinical staff, 4 psychiatrists, 11 physicians, 3 informatics specialists and 2 physician leaders. **Results:** Preliminary analysis shows that providers' outcome expectancy was statistically significantly different in the technology-facilitated group. Additionally, providers felt they could more consistently monitor adherence and side effects of treatment and adjust therapy accordingly. **Conclusions:** Technology facilitated depression care screening and monitoring, if adopted by providers, may be an effective method of improving care for low-income Latino diabetic patients. This analysis found providers adopted the technology and changed their practice to be guideline-concordant. This adoption has increased their outcome expectancy of safety-net patients.

Poster Abstracts

207

Tcfap2c Regulates Expression of *Mmp9* and May Mediate Cancer Invasion and Metastasis in Her2-Positive Breast Tumors

T Wu*, **JM Park****, **GW Woodfield****, **AR Cyr****, **J De Andrade****, **W Zhang****, **FE Domann****, **RJ Weigel***
**Department of Surgery, University of Iowa, Iowa City, IA*

Introduction: Human transcription factor TFAP2C and its mouse homolog TCFAP2C play a critical role in mammary gland development. Previously published data has shown a positive association between levels of AP-2 proteins and matrix metalloproteinase 9 (MMP9) in primary human breast cancers. We sought to clarify the role of *Tcfap2c* in regulation of *Mmp9* expression and its effect on invasion in breast cancer. **Methods:** Transgenic mice expressing an activated MMTV-*Neu* gene were crossed with animals with a floxed *Tcfap2c* gene. RT-PCR was used to analyze RNA expression. Protein expression was determined by Western blot. Chromatin immunoprecipitation-sequencing (ChIP-Seq) was used to identify genomic binding sites of TCFAP2C in the mouse cells. Tumors and tissues were analyzed by hematoxylin and eosin (H&E). Matrigel invasion assays were performed *in vitro*. **Results:** A primary mouse breast cancer cell line was derived from tumors obtained from animals harboring a mouse mammary tumor virus-driven (MMTV) rat activated-*Neu* oncogene and homozygous floxed *Tcfap2c*. The effect of knockout (KO) of *Tcfap2c* was assessed by comparing parallel cell lines created by infection with Ad-*Cre* or Ad-Empty (control); western blot confirmed loss of TCFAP2C expression with Ad-*Cre*. Loss of *Tcfap2c* decreased *Mmp9* RNA expression to 8% of control cells ($p<0.05$). ChIP-Seq revealed at least five significant TCFAP2C peaks within the *Mmp9* regulatory regions. Tumor invasiveness demonstrated a 48% reduction in Matrigel invasion with KO of *Tcfap2c* ($p<0.008$). Transgenic *Neu*-expressing animals were created with conditional KO of *Tcfap2c* expressing MMTV-*Cre* compared to *Cre*-negative animals. Animals were assessed for lung metastases in both genetic backgrounds when mammary tumors reached 2 cm. No lung metastases were identified in the KO background compared to 75% with metastatic disease in the control animals ($p<0.05$). **Conclusion:** *Tcfap2c* induced *Mmp9* expression in *Neu*-activated mouse mammary carcinomas. Critically, loss of *Tcfap2c* appears to attenuate tumor invasion and lung metastasis in HER2-positive breast tumors. Further analysis of the downstream targets of TCFAP2Cmay yield additional insight into novel means of treating HER2-positive breast cancer.

208

Generation of Pluripotent Stem Cells from Patients with Familial Dilated Cardiomyopathy Due to a Novel RBM20 Mutation

SP Wyles, **R Beraldi, FJ Secreto, G Hawse, T Olson, TJ Nelson**
Center for Regenerative Medicine, Mayo Clinic, Rochester, MN

Dilated cardiomyopathy (DCM) is a leading cause of heart failure with an estimated prevalence of 36.5 per 100,000 individuals. Currently, it is the most common indication for heart transplantation and is associated with substantial mortality. Recently, genetic linkage analysis in families with autosomal-dominant DCM led to the discovery of the distinct heterozygous missense mutation in the highly conserved arginine/serine (RS)-rich region of exon 9, known as ribonucleic acid binding motif protein 20 (RBM20). This particular mutation is strongly associated with morbidity and mortality. Pluripotent stem cells generated from patients with RBM20-associated DCM would be useful in disease modeling to better understand the underlying pathology and to develop clinical-grade therapeutics. In this study, skin fibroblasts from RBM20 mutation-associated DCM patients and controls were derived from explants of 3-mm dermal biopsies after informed consent under approved protocols by Mayo Clinic. We show here that induced pluripotent stem cells (iPSCs) can be generated from patients with DCM by reprogramming their adult fibroblasts with four transcription factors (Sox2, Oct4, Klf4, and c-Myc). RBM20-DCM-specific iPSCs have the hallmarks of pluripotency as shown by immunofluorescence analysis, gene-specific quantitative PCR, spontaneous differentiation into embryoid bodies, and karyotyping. These results are a step toward using iPSCs for hereditary DCM disease modeling, as well as providing the foundation to bioengineer gene therapy approaches in the era of individualized medicine.

209

Transcription Factor ZBP-89 Protects the Colon from Inflammation-induced Tumorigenesis

Alexander Yang, **Bryan Essien, and Juanita L. Merchant**
Department of Internal Medicine, Division of Gastroenterology, Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor

Background: The Kruppel-type transcription factor Zinc-finger Binding Protein-89 (ZBP) expression is regulated by butyrate, and forms protein complexes with known tumor suppressor factor, e.g., p53, p300, ataxia-telangiectasia mutated (ATM). To study the role of ZBP-89 in vivo, we generated a conditional knockout of the transcription in the intestine and colon. **Aim**: To determine whether ZBP-89 modulates β-catenin transcription activity and consequently affects tissue homeostasis and transformation. **Methods**: Mice conditional null for ZBP-89 (ZBP-89$^{\Delta Int}$) and WT littermate were injected intraperitoneally with 7.4mg/kg axomymethane (AOM). Water containing 2% dextran sulfate sodium (DSS) was administered in day 5 for 5 days followed by 7 days of untreated water for recovery. The protocol was repeated thrice and mice were sacrificed 5 weeks later for tumor evaluation, mRNA analysis and histology. Gene expression levels for cyclinD1, c-myc and Axin2 were determined by rt-qPCR on the colonic tissue. We also co-transfected ZBP-89 expression vector with and without β-catenin into HEK293 and SW480 human colorectal cancer cell lines in addition to TCF reporter plasmid TOPFLASH to assess direct regulation of Wnt-β-catenin-TCF transcriptional activity. Total lysates of SW480 was used to performed co-immunoprecipitation to demonstrate the association of ZBP-89 with β-catenin. Confocal microscopy was also used to demonstrate that ZBP-89 co-localized with β-catenin in the nucleus. **Results**: We observed 100% increase in tumor incidence and size ZBP-89$^{\Delta Int}$ in the compared to WT mice after the administration of AOM/DSS, N= 34 mice/group. The tumors were flat non-invasive adenoma located in the distal colon rectum

in both WT and ZBP-89$^{\Delta Int}$ mice. The mRNA of cyclinD1, c-myc and Axin2 were significantly increase in the ZBP-89$^{\Delta Int}$ compared to WT mice. Ectopic expression of ZBP-89 mitigates the transcription activity of β-catenin in HEK293 and SW480 cell lines. Co-immunoprecipitation shows that ZBP-89 and β-catenin form a complex. We observed nuclear co-localization of ZBP-89 and β-catenin. **Conclusion**: ZBP-89 appears to suppress the β-catenin-TCF transcription complex by forming protein-protein interaction with β-catenin, providing a possible mechanism by which this transcription factor inhibits colonic tumor formation.

210

RNAi-mediated Inhibition of Hepatic ALAS1 Provides Effective Prevention and Treatment of Induced Acute Attacks in Acute Intermittent Porphyria Mice

***M Yasuda*[1], L Gan[1], C Yu[1], W Querbes[2], A Liebow[2], K Fitzgerald[2], and RJ Desnick[1]**

[1]Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY; [2]Alnylam Pharmaceuticals, Cambridge, MA

The acute hepatic porphyrias include four inherited disorders of heme biosynthesis that are characterized by life-threatening acute neurovisceral attacks. Various factors that increase the expression of hepatic 5-aminolevulinic acid synthase 1 (ALAS1) - the first and rate-limiting enzyme in the heme biosynthetic pathway - result in the marked accumulation of the neurotoxic porphyrin precursors, 5-aminolevulinic acid (ALA) and porphobilinogen (PBG), and precipitate the acute attacks. Currently, the acute attacks are treated with a series of intravenous hemin infusions, but a faster-acting and safer therapy is desirable. Therefore, we designed a liver-directed RNA interference (RNAi) therapeutic using small interfering RNAs (siRNAs) specifically targeting *Alas1* (designated Alas1-siRNAs) and evaluated its effectiveness following intravenous administration in a mouse model of acute intermittent porphyria (AIP), the most common acute hepatic porphyria. These mice have slightly elevated baseline urinary and plasma ALA and PBG levels which are markedly increased by administration of the porphyrinogenic drug phenobarbital, thereby inducing a "biochemical" acute attack. To identify a potent Alas1-siRNA, >40 siRNAs targeting the *Alas1* gene were screened for their ability to reduce *Alas1* mRNA expression in cultured hepatic cells. The most active compounds were formulated into lipid nanoparticles for efficient hepatic delivery and evaluated for their ability to downregulate hepatic *Alas1* mRNA in wild-type mice. A single prophylactic administration of the optimal Alas1-siRNA in the AIP mice prevented the phenobarbital-induced acute attacks for approximately two weeks. When Alas1-siRNA was infused during an induced acute attack, plasma ALA and PBG levels decreased within 8 to 12 hours, much more rapidly and effectively than a single hemin infusion. Alas1-siRNA was well tolerated and a single therapeutic dose did not deplete hepatic 'free' heme. Further, subcutaneous injection of a hepatocyte *N*-acetylgalactosamine (*GalNAc*) receptor-targeted Alas1-siRNA formulation was effective in preventing the phenobarbital-induced acute attacks, thereby facilitating convenient therapeutic delivery. These preclinical studies provide proof-of-concept for the development of siRNA-mediated therapy to prevent and treat the acute attacks in the acute hepatic porphyrias.

This research was funded by a grant from Alnylam Pharmaceuticals, for which MY and RJD are consultants.

211

De Novo Mutations in Histone Modifying Genes in Congenital Heart Disease

***Samir Zaidi*[1,3], Murim Choi[1,3], Andrew Robson[2], Svetlana Makova[2], Jonathan Seidman[4], Wendy K. Chung[5], Bruce D. Gelb[6], Elizabeth Goldmuntz[7], Christine E. Seidman[4], Mustafa Khokha[2], Martina Brueckner[2], and Richard Lifton[1,3]**

[1]Department of Genetics, of [2]Pediatrics, and [3]HHMI, Yale University, New Haven; [4]Department of Genetics and HHMI, Harvard Medical School, Boston; [5]Departments of Pediatrics and Medicine, Columbia University, New York; [6]Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York; [7]Department of Pediatrics, Perelman School of Medicine, Philadelphia, PA

Severe congenital heart disease (CHD), the most common cause of non-infectious death in newborns, often occurs sporadically and impairs reproductive fitness. This suggests a role for *de novo* mutations in its etiology. We compared the incidence of *de novo* mutations in 362 severe CHD cases and 264 controls by analyzing exome sequences of parent-offspring trios (Zaidi *et al., Nature*, 2013). We first partitioned genes into the top 25% (high heart expression, HHE) and bottom 75% (low heart expression, LHE) of expression in e14.5 mouse heart. CHD cases showed a significant excess of protein-altering *de novo* mutations in HHE genes with an odds ratio that progressively increased from 2.53 (P=0.003) for protein-altering mutations, to 3.67 (P=0.0004) for damaging mutations and protein-altering mutations at highly conserved positions, to 7.5 (P=0.01) for damaging mutations alone. Importantly, we found that genes involved in production, removal or reading of H3K4 methylation, or ubiquitination of H2BK120, which is required for H3K4 methylation were significantly increased (P=0.0004). H3K4 and H3K27 methylation mark 'poised' promoters and enhancers that regulate expression of key developmental genes. Mutated genes included components of an H3K4 methylase (*MLL2* and *WDR5*), H3K4 demethylases (*KDM5A* and *KDM5B*), and the H3K4 'reader' *CHD7*. Three genes that ubiquitinate (*RNF20, UBE2B*) or de-ubiquitinate (*USP44*) H2BK120 were also mutated. Two *de novo* mutations were noted in *SMAD2* that induces H3K27 demethylation. We estimate that *de novo* point mutations in several hundred genes collectively contribute to ~10% of severe CHD. We are functionally characterizing these genes using the *Xenopus* heart, which allows the rapid study of gene dosage on specific stages of cardiac morphogenesis. Knocking down *RNF20* in *Xenopus* embryos using morpholinos resulted in profound cardiac and cilia motility defects, recapitulating the missing inner dynein arm in a CHD patient with an *RNF20* mutation. Thus, we not only implicate *de novo mutations* in histone-modifying genes in the etiology of CHD, but also establish a role for *RNF20* mutations in causing the ciliary dysfunction that underlies defective cardiac development.

Poster Abstracts

212

Improving the Efficiency of Abdominal Aortic Aneurysm Wall Stress Computations

JE Zelaya**[*]****, S Goenezen[†], PT Dargon[*], AF Azarbal[*], S Rugonyi[*]**
[*]Oregon Health & Science University, Portland, OR; [†]Texas A&M, College Station, TX

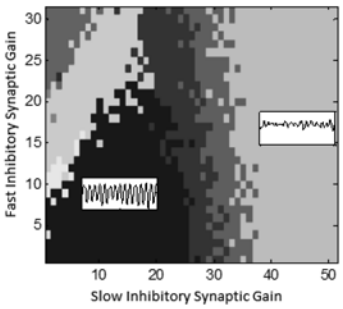
An abdominal aortic aneurysm (AAA) is a pathological dilation of the abdominal aorta that carries a high mortality rate if ruptured. Aneurysmal stability is commonly determined in the clinic by measuring the maximum transverse diameter of the aneurysm, a marker that is not sensitive and specific enough for assessing rupture risk. Recent studies have found that wall stress, a force per unit area, is a more accurate predictor of AAA rupture. Wall stress is typically computed using models of patient-specific AAA geometries extracted from computed tomography (CT) scans, and finite element analysis, a numerical method. These computations are currently intensive, in part, because tissue walls are conventionally modeled with nonlinear mechanical properties; hence, the use of finite element patient-specific models to efficiently assess rupture risk in the clinic is currently a challenge. To facilitate wall stress computations, we modeled AAA walls using a linear model approach, which involves characterizing aneurysmal tissue with linear mechanical properties. To assess the accuracy of our approach, we compared wall stresses computed using linear models of simple axisymmetric and patient-specific AAAs to the stresses of reference AAA models. Comparisons were also conducted between the wall stresses of conventional AAA models, in which the walls were simulated using nonlinear properties, and the reference model stresses. For simple axisymmetric AAAs, the reference models were generated from hypothetical initial configurations assumed to be truly unloaded with known nonlinear mechanical properties. These assumptions were also applied to the initial configurations of patient-specific AAA models with the exception that the initial geometries were extracted from CT scan images rather than being hypothetically constructed. Application of transmural pressures on the initial configurations of the reference models resulted in deformed geometries with wall stresses, which served as a comparative baseline for the study. Compared to the conventional model, we have shown that our linear model approach yields relatively accurate wall stresses for aneurysms of various sizes, thicknesses, transmural pressures and material properties. These results suggest that the linear approach has great potential for yielding patient-specific wall stresses with reliable accuracy in an efficient manner. Improving the accuracy and efficiency of wall stress computations is crucial for identifying patients in need of urgent repair. Thus, the linear model may be a promising clinical tool that may aid clinicians in making effective decisions about AAA management.

213

Classification of EEG States through the Assimilation of Data to a Computational Neural Population Model

JL Zick, **TI Netoff**
University of Minnesota, Minneapolis, MN

Interpretation of human electroencephalogram (EEG) studies is largely qualitative and requires the expertise of highly trained professionals. Relatively simple computational neuronal population models can produce EEG-like activity and can provide insight into underlying mechanisms that generate the signal. Our goal is to use these models to develop a robust method for quantitatively classifying EEG data. Using a neurophysiologically relevant model of neuronal populations, we are able to produce various types of EEG activity such as normal background activity, slow rhythmic oscillations, sporadic spikes, and others depending on the model parameters, as can be seen in the figure. In our model, the parameters of interest represent excitatory and inhibitory synaptic gains. Our goal is to fit the model to human EEG data, then use the model to classify the EEG. Data assimilation is the process of fitting a model to actual data, and has been used extensively in weather forecasting and other areas but is relatively new to neuroscience. The process involves comparing EEG voltage values to model outputs, then varying parameter values recursively until the model produces behaviors similar to those seen in the EEG. Once the model has been fit to the data, we can use the corresponding parameter values to classify the data into one of the types of behaviors. This work has the potential to be used as an objective automatic classification system that can assist neurologists in interpreting EEGs.



Various combinations of model parameters result in different behaviors. Plot of the parameter space of our model for a fixed value of the excitatory gain parameter and a range in values of two types of inhibitory gains. Colors represent different classes of behaviors. Two types of behaviors are shown associated with the regions of parameter space that produce them.

Oral Presentations Author Index

Poster Author Index

Poster Author Index

Poster Author Index

Poster Author Index

Poster Author Index

Poster Author Index

Poster Author Index

Poster Author Index

Poster Author Index

Poster Author Index

T

Poster Author Index

Poster Author Index

Notes

The Harrington Prize for Innovation in Medicine

Applications accepted through August 15, 2014.

The Harrington Prize for Innovation in Medicine, presented by the American Society for Clinical Investigation (ASCI) and the Harrington Discovery Institute at University Hospitals Case Medical Center, honors the physician-scientist who has moved science forward with notable achievements in innovation, creativity and potential for clinical application.

Applications are now being accepted for the 2015 Harrington Prize – an international award open to those holding an MD or equivalent degree. This annual prize includes:

– An unrestricted $20,000 honorarium
– The Harrington Prize Lecture, delivered at the annual meeting of the ASCI
– A review, published in the Journal of Clinical Investigation

To learn more or to apply, visit HarringtonDiscovery.org/Prize

THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION
honoring the physician-scientist

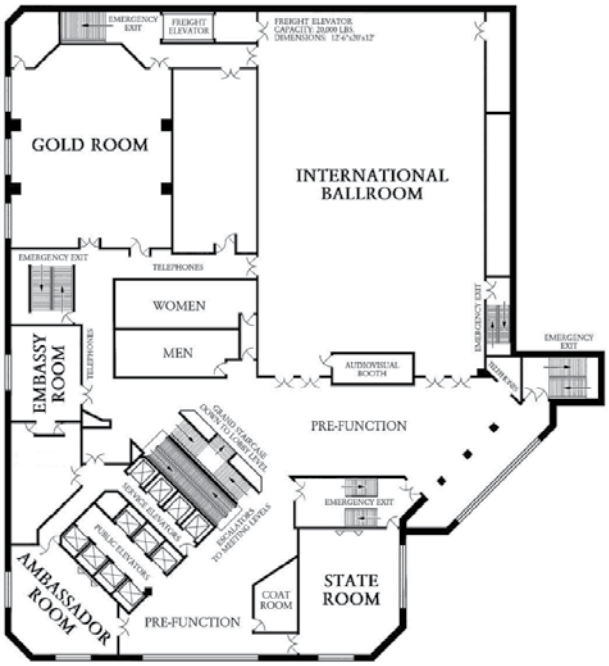
THE HARRINGTON PROJECT
FOR DISCOVERY & DEVELOPMENT

Cleveland | Ohio

Among the nation's leading academic medical centers, University Hospitals Case Medical Center is the primary affiliate of Case Western Reserve University School of Medicine, a nationally recognized leader in medical research and education.

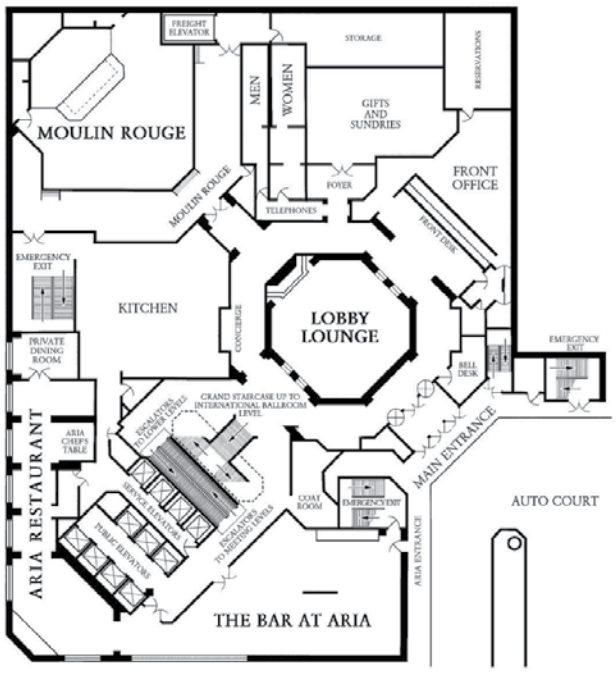
Hotel Floor Plans

International Ballroom Level (2nd level)

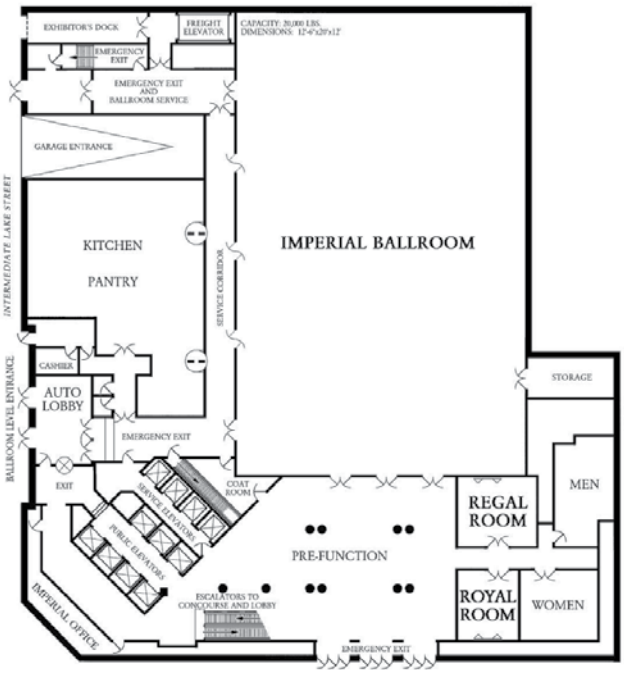


Meeting Room Level (3rd level)

Lobby Level (1st level)



Imperial Ballroom Level (B2)



ASCI / AAP Joint Meeting

Thanks This Year's Generous Supporters

Foundation Donors

DORIS DUKE CHARITABLE FOUNDATION

BURROUGHS WELLCOME FUND

Academic Benefactors

Northwestern Medicine®

RUSH UNIVERSITY

THE HARRINGTON PROJECT FOR DISCOVERY & DEVELOPMENT

Academic Supporter

SCHOOL OF MEDICINE
CASE WESTERN RESERVE UNIVERSITY

Academic Friend

OMRF