# Meeting Program and Abstracts

# ASCI / AAP Joint Meeting 2013

## April 26 – 28, 2013

The Fairmont Chicago
Chicago, Illinois

American Society for Clinical Investigation 1908

APSA
American Physician Scientists Association

www.jointmeeting.org

# Special Events at the 2013 ASCI/AAP Joint Meeting

## Friday April 26

### ASCI President's Reception
**(by invitation only)**

**6:00 – 7:00 p.m.**
**Gold Room (2nd Level)**

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

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

**Clinical Research: The Uphill Climb**
**Speaker: David G. Nathan, MD**
*Harvard Medical School, Dana-Farber Cancer Institute*

### 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**
**Skydeck at the Willis (Sears) Tower**

**Speaker: Dania Daye, MD, PhD Candidate**
*HHMI-NIBIB Interfaces Scholar,*
*University of Pennsylvania School of Medicine*

## Saturday April 27

### AAP Annual Reception & Dinner
**7:00 – 10:00 p.m.**
**Imperial Foyer & Ballroom (Level B2)**

**A Funny Thing Happened on the Way to Stockholm**
**Speaker: Robert Lefkowitz, MD**
*Duke University*

### APSA Dinner
**7:30 - 9:00 p.m.**
**Moulin Rouge, 1st Floor**

**Don't Get Bit: How Expeditions Drive Clinical Research**
**Speaker: Matthew Lewin, MD, PhD**
*Center for Exploration and Travel Health,*
*California Academy of Sciences*

### Dessert Reception
**(open to all attendees)**

**10:00 p.m. – Midnight**
**Imperial Foyer**

APSA
American Physician Scientists Association

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# General Information

## Accreditation and Credit Designation

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

The University of Chicago Pritzker School of Medicine designates this live activity for a maximum of **9** *AMA PRA Category 1 Credits*™. Physicians should claim only the credit commensurate with the extent of their participation in the activity. Nurses and other health professionals will receive a Certificate of Attendance. For information on applicability and acceptance, please consult your professional licensing board.

### Learning Objectives

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

The 2013 ASCI/AAP Joint Meeting program will feature lectures by accomplished researchers who will discuss state-of-the-art advances in their respective fields. The program is designed to foster in-depth discussions and close interactions among the meeting participants.

## Meeting Evaluation

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

## Poster Session Schedule

### Friday, April 26

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

### Saturday, April 27

| | |
|---|---|
| **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.** A faculty member will visit posters during this time to discuss your work.

### Best Poster Awards

Best Poster Awards will be given in the amount of $1,000 each. Members of the ASCI, AAP, and APSA will judge posters on scientific novelty, quality and clarity of presentation. **Awards will be presented on Sunday, April 28, from 8:20 – 8:30 a.m.**

### ASCI Membership Desk

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

### AAP Membership Desk

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

### Registration Hours

| | |
|---|---|
| **Friday, April 26** | 7:00 a.m. – 7:00 p.m. |
| **Saturday, April 27** | 7:00 a.m. – 5:00 p.m. |
| **Sunday, April 28** | 7:00 a.m. – 10:00 a.m. |

# Scientific Program

## Friday, April 26

| Time | Event | Location |
|---|---|---|
| 7:00 a.m. – 7:00 p.m. | Registration | International Ballroom Foyer |
| 8:30 a.m. – 12:30 p.m. | APSA Business Meeting | Moulin Rouge |
| 1:00 p.m. – 3:00 p.m. | Poster Setup | Imperial Ballroom |
| 12:30 p.m. – 3:30 p.m. | **APSA Plenary Session** | Moulin Rouge |
| 12:30 p.m. – 1:00 p.m. | **APSA Keynote – Debra Houry, MD, MPH**<br>*Emory University School of Medicine*<br>**Public Scholarship and the Role of Scientists** | |
| 1:00 p.m. – 1:30 p.m. | **APSA Keynote – Jonathan Epstein, MD**<br>*Perelman School of Medicine, University of Pennsylvania*<br>**Cardiac Growth and Regeneration** | |
| 2:00 p.m. – 3:00 p.m. | **APSA Session I: Women in Medicine Panel**<br>Moderator: **Jill Baren, MD,**<br>*Perelman School of Medicine, University of Pennsylvania*<br>Panelists:<br>**Gail Tomlinson, MD, PhD,** *University of Texas Medical School at San Antonio*<br>**Juliane Bubeck Wardenburg, MD, PhD,** *University of Chicago School of Medicine*<br>**Melanie Thomas, MD, MS,** *Medical University of South Carolina College of Medicine* | |
| 3:00 p.m. – 3:30 p.m. | **APSA Keynote – Levi A. Garraway, MD, PhD**<br>*Harvard Medical School, Dana-Farber Cancer Institute*<br>**The Cancer Genome in Biology and Therapy** | |
| 3:30 p.m. – 6:00 p.m. | **Plenary Session I –**<br>**Understanding Disease Mechanisms to Improve Human Health**<br>Moderators: **Warner C. Green, Peter Tontonoz and Taylor Heald Sargent** | International Ballroom |
| 3:30 p.m. – 4:00 p.m. |  **Francis Collins, MD, PhD**<br>*National Institutes of Health*<br>**Scaling up the Potential of Clinical Research** | |
| 4:00 p.m. – 4:30 p.m. |  **Charles Rice, PhD**<br>*Rockefeller University*<br>**Hepatitis C: Is the End in Sight?** | |
| 4:30 p.m. – 4:45 p.m. | **Presentation of Career Development Awards** | |
| 4:45 p.m. – 5:15 p.m. |  **Eric Verdin, MD**<br>*Gladstone Institutes, University of California, San Francisco*<br>**Mitochondrial Protein Acylation and Metabolic Regulation** | |
| 5:15 p.m. – 5:45 p.m. |  **Shinya Yamanaka, MD, PhD**<br>*Center for iPS Cell Research and Application (CiRA), Japan, and Gladstone Institutes (UCSF)*<br>**Induction of Pluripotency by Defined Factors** | |
| 5:45 p.m. – 6:00 p.m. | Q&A | |
| 6:00 p.m. – 7:00 p.m. | **ASCI President's Reception**<br>(by invitation only) | Gold Room (2nd Level) |
| 6:00 p.m. – 9:30 p.m. | Poster Viewing | Imperial Ballroom |

# Scientific Program

## Friday, April 26

| Time | Event | Location |
|---|---|---|
| 7:00 p.m. – 9:00 p.m. | **AAP President's Dinner**<br>(by invitation only) | Mid-America Club |
| 7:00 p.m. – 9:00 p.m. | **ASCI Dinner & New Member Induction Ceremony**<br>(ticketed guests only)<br>Speaker: **David G. Nathan, MD**<br>*Harvard Medical School, Dana-Farber Cancer Institute*<br>**Clinical Research: The Uphill Climb** | Moulin Rouge (1st Level) |
| 7:00 p.m. – 9:00 p.m. | **APSA Dinner Outing**<br>(On Your Own)<br>Sign up at the Registration Desk outside the International Ballroom | Off Site |
| 9:00 p.m. – Midnight | **APSA Welcome Reception & Presidential Address**<br>Speaker: **Dania Daye, MD, PhD Candidate**<br>*HHMI-NIBIB Interfaces Scholar, University of Pennsylvania School of Medicine* | Skydeck<br>Willis (Sears) Tower |

## Saturday, April 27

| | | |
|---|---|---|
| 7:00 a.m. – 5:00 p.m. | Registration | International Ballroom Foyer |
| 7:00 a.m. – 8:15 a.m. | Young Investigators' Mentoring Breakfast | Moulin Rouge |
| 8:15 a.m. – 11:45 a.m. | **Plenary Session II –**<br>**Understanding Disease Mechanisms to Improve Human Health**<br>Moderators: **J. Larry Jameson, William Hahn and Dania Daye** | International Ballroom |
| 8:15 a.m. – 8:45 a.m. |  <br>**Kevin Shannon, MD**<br>*University of California, San Francisco*<br>**Reflections of a Former MSTP Director on Physician/ Scientist Training** | |
| 8:45 a.m. – 9:15 a.m. |  <br>**Charles Sawyers, MD**<br>*Memorial Sloan-Kettering Cancer Center*<br>**Developing New Prostate Cancer Drugs** | |
| 9:15 a.m. – 9:30 a.m. | **APSA Trainee Presentation**<br>**Leo Y. Luo, BS**<br>*Broad Institute of Harvard and MIT*<br>**Role of FRS2 and FGF/FGFR Autocrine Signaling in the Proliferation of Ovarian Cancer Cells** | |
| 9:30 a.m. – 10:00 a.m. |  <br>**Deepak Srivastava, MD**<br>*Gladstone Institutes, University of California, San Francisco*<br>**Direct Cardiac Reprogramming:**<br>**From Developmental Biology to Regeneration** | |
| 10:00 a.m. – 10:30 a.m. | Break | International Foyer |
| 10:30 a.m. – 11:00 a.m. |  <br>**Barbara Kahn, MD**<br>*Harvard Medical School,*<br>*Beth Israel Deaconess Medical Center*<br>**Novel Mechanisms by Which Adipose Tissue Regulates Systemic Insulin Sensitivity and the Risk for Diabetes** | International Ballroom |
| 11:00 a.m. – 11:15 a.m. | **APSA Trainee Presentation**<br>**InYoung Kim, PhD**<br>*University of Chicago*<br>**Heat Shock Protein 70 Demonstrates IL-10 Mediated Immune Modulation in Experimental Colitis** | |

# Scientific Program

## Saturday, April 27

| Time | Event | Location |
|---|---|---|
| 11:15 a.m. – 11:45 a.m. |  **Stanley Prusiner, MD**<br>*University of California, San Francisco*<br>**Unifying Role for Prions in Degenerative Diseases** | International Ballroom |
| 11:45 a.m. – 1:30 p.m. | Poster Session with Lunch | Imperial Ballroom and Foyer |
| 1:30 p.m. – 2:00 p.m. | Poster Dismantle | Imperial Ballroom |
| 1:30 p.m. – 3:15 p.m. | **APSA Breakout Sessions** | |
| 1:30 p.m. – 2:15 p.m. | **Residency 101**<br>Moderator: **Michael (Kerry) O'Banion, MD, PhD**<br>*University of Rochester School of Medicine*<br>Panelists:<br>**Dave Scoville, MD, PhD,** *Stanford Integrated Cardiothoracic Surgery*<br>**James Liao, MD,** *University of Chicago*<br>**Nicole Grieselhuber, MD, PhD,** *The Ohio State University Internal Medicine* | Gold Room |
| 1:30 p.m. – 2:15 p.m. | **Writing for Basic Science & Clinical Journals**<br>Moderator: **David Markovitz, MD,** *University of Michigan*<br>Panelists:<br>**Howard Rockman, MD,** *Editor-in-Chief, JCI*<br>**Howard Bauchner, MD,** *Editor-in-Chief, JAMA* | Crystal Room |
| 1:30 p.m. – 4:10 p.m. | **ASCI and AAP New Member Presentations**<br>Moderators: **Paul Rothman, Mukesh Jain and Evan Noch** | International Ballroom |
| 1:30 p.m. – 1:50 p.m. | **Mary Armanios, MD (New ASCI Member)**<br>*Johns Hopkins University School of Medicine*<br>**Telomerase and Idiopathic Pulmonary Fibrosis** | |
| 1:50 p.m. – 2:10 p.m. | **Susan Quaggin, MD (New AAP Member)**<br>*Northwestern University*<br>**VEGF and the Glomerular Barrier — Two Sides to the Story** | |
| 2:10 p.m. – 2:30 p.m. | **Miriam Merad, MD, PhD (New ASCI Member)**<br>*Mount Sinai School of Medicine*<br>**Regulation of the Dendritic Cell and Macrophage Lineage** | |
| 2:30 p.m. – 2:50 p.m. | **Joseph Gleeson, MD (New AAP Member)**<br>*University of California, San Diego*<br>**Treating the Untreatable:**<br>**An Exome Sequencing Approach in Neurodevelopmental Disease** | |
| 2:30 p.m. – 3:15 p.m. | **APSA Policy Panel**<br>Moderator: **Barry Coller, MD,** *Rockefeller University*<br>Panelists:<br>**Mike Bristow, MD, PhD,** *University of Colorado*<br>**Anada Chakrabarty, PhD,** *University of Illinois, Chicago*<br>**Francis Collins, MD, PhD,** *NIH* | Gold Room |
| 3:00 p.m. – 3:30 p.m. | Break | International Ballroom Foyer |

# Scientific Program

## Saturday, April 27

| Time | Event | Location |
|---|---|---|
| 3:30 p.m. – 3:50 p.m. | **Richard James Gilbertson, MD, PhD (New ASCI Member)**<br>*St. Jude Children's Research Hospital*<br>**Mapping the Origins of Cancer** | International Ballroom |
| 3:50 p.m. – 4:10 p.m. | **David Valle, MD (New AAP Member)**<br>*Johns Hopkins University School of Medicine*<br>**The Search for Disease Genes: What Mendel Can Tell us About Medicine** | International Ballroom |
| 4:10 p.m. – 4:45 p.m. | **APSA Keynote – Shannon Kenney, MD**<br>*University of Wisconsin-Madison School of Medicine and Public Health*<br>**Epstein-Barr Virus From Bench to Bedside** | International Ballroom |
| 4:45 p.m. – 5:00 p.m. | **APSA Trainee Presentation**<br>**Stephanie R. Jackson, BSE, MS**<br>*Saint Louis University School of Medicine*<br>**T-bet Dictates CD8+ T Cell Tolerance Versus Immunity Following Antigen Recognition** | International Ballroom |
| 5:00 p.m. – 5:30 p.m. |  **AAP Presidential Address: Warner C. Greene, MD, PhD**<br>*Gladstone Institutes, University of California, San Francisco*<br>**Seeking Sustainable Solutions to Global Health Challenges: No More Band-Aids** | |
| 5:30 p.m. – 6:00 p.m. |  **ASCI Presidential Address: William C. Hahn, MD, PhD**<br>*Harvard Medical School, Dana-Farber Cancer Institute*<br>**The Perfect Storm: Challenges and Opportunities for Translational Research** | |
| 7:00 p.m. – 10:00 p.m. | **AAP Annual Reception and Dinner**<br>**Robert Lefkowitz, MD**<br>*Duke University*<br>**A Funny Thing Happened on the Way to Stockholm** | Imperial Ballroom |
| 7:30 p.m. – 9:00 p.m. | **APSA Dinner**<br>Speaker: **Matthew Lewin, MD, PhD**<br>*Center for Exploration and Travel Health, California Academy of Sciences*<br>**Don't Get Bit: How Expeditions Drive Clinical Research** | Moulin Rouge |
| 10:00 p.m. – Midnight | **Dessert Reception** (open to all attendees) | Imperial Ballroom Foyer |

## Sunday, April 28

| | | |
|---|---|---|
| 7:00 a.m. – 5:00 p.m. | Registration | International Ballroom Foyer |
| 7:00 a.m. – 8:15 a.m. | Young Investigators' Mentoring Breakfast | Moulin Rouge |
| 8:00 a.m. – Noon | **Plenary Session III –**<br>**Understanding Disease Mechanisms to Improve Human Health**<br>Moderators: **Warner C. Greene, William C. Hahn and Katherine Hartmann** | International Ballroom |
| 8:00 a.m. – 8:20 a.m. | AAP Business Meeting | |

# Scientific Program

## Sunday, April 28

| Time | Event | Location |
|---|---|---|
| 8:20 a.m. – 8:30 a.m. | **Best Poster Award Presentation** | International Ballroom |
| 8:30 a.m. – 9:15 a.m. | **Kober Medal Presentation**<br>  <br>Recipient: **John T. Potts, Jr., MD**<br>*Massachusetts General Hospital*<br>  <br>Presenter: **J. Larry Jameson, MD, PhD**<br>*Perelman School of Medicine, University of Pennsylvania* | |
| 9:15 a.m. – 10:00 a.m. |  <br>**ASCI/Stanley J. Korsmeyer Award Lecture**<br>**Bruce Beutler, MD**<br>*UT Southwestern Medical Center* | |
| 10:00 a.m. – 10:45 a.m. |  <br>**Stanley Hazen, MD, PhD**<br>*Cleveland Clinic*<br>**Targeting the Gut to Treat the Heart** | |
| 10:45 a.m. – 11:00 a.m. | **APSA Trainee Presentation**<br>**Lulu Sun, BSc**<br>*Washington University in Saint Louis University School of Medicine*<br>**Systemic Type I Interferons Indirectly Promote Epithelial Proliferation and Turnover** | International Ballroom |
| 11:00 a.m. – 11:15 a.m. | **APSA Trainee Presentation**<br>**Samuel D. Quaynor, BA**<br>*Georgia Health Sciences University*<br>**Decreased Puberty and Fertility Development in NELF KO Mice Due to Impaired GNRH Neuron Migration** | |
| 11:15 a.m. – Noon | **APSA Keynote**<br>**Vivian S. Lee, MBA, MD, PhD**<br>*University of Utah*<br>**MRI: From Science to Society** | |
| Noon – 1:00 p.m. | **APSA Post Graduate Opportunities Panel: Industry, Government, Academia**<br>Moderator: **Lawrence (Skip) Brass, MD, PhD**<br>*Perelman School of Medicine, University of Pennsylvania*<br>Panelists:<br>**Sapan Desai, MD, PhD, MBA, CEO and president**<br>*Surgisphere Corporation; University of Texas at Houston; Duke University*<br>**Griffin Rodgers, MD, MBA**<br>*National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)*<br>**Dianna Milewicz, MD, PhD**<br>University of Texas Medical School at Houston | |
| 1:00 p.m. – 2:30 p.m. | APSA Residency Luncheon | Moulin Rouge |

# Committee and Faculty

## 2013 ASCI/AAP Joint Meeting Planning Committee

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

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

**David A. Brenner, MD**
AAP Immediate Past President
*University of California, San Diego*

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

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

**Elizabeth McNally, MD**
ASCI Immediate Past President
*The University of Chicago*

## ASCI Council

### Officers

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*Harvard Medical School, Dana-Farber Cancer Institute*

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

Vice President
**Mukesh K. Jain, MD**
*Case Western Reserve University School of Medicine*

Secretary-Treasurer
**Theodora S. Ross, MD, PhD**
*The University of Texas Southwestern Medical Center at Dallas*

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

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**Vivian G. Cheung, MD**
*Children's Hospital of Philadelphia*

**Mark Gladwin, MD**
*University of Pittsburgh School of Medicine*

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

**Judith A. James, MD, PhD**
(Secretary-Treasurer Elect)
*Oklahoma Medical Research Foundation*

**Brendan Lee, MD, PhD**
*Baylor College of Medicine*

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

## AAP Council

### Officers

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*Gladstone Institute of Virology & Immunology*

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*Stanford University*

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

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*University of Michigan*

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

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

**Kenneth Kaushansky, MD**
*Stony Brook University School of Medicine*

**Gary Koretzky, MD, PhD**
*Abramson Family Cancer Research Institute/University of Pennsylvania*

**Stanley Lemon, MD**
*University of North Carolina at Chapel Hill*

**Paul Rothman, MD**
*Johns Hopkins University School of Medicine*

**Charles Sawyers**
*Memorial Sloan-Kettering Cancer Center*

**Christine Seidman, MD**
*Brigham and Women's Hospital*

**Stefan Somlo, MD**
*Yale School of Medicine*

# 2013 Career Development Award Recipients

**G. Brandon Atkins**
*Case Western Reserve University*

**Monica P. Goldklang**
*Columbia University*

**Wenyu Huang**
*Northwestern University*

**Heather K. Morris**
*Columbia University Medical Center*

**Naoki Sawada**
*Tokyo Medical and Dental University*

**John P. Shen**
*University of California, San Diego*

**Elizabeth K. Speliotes**
*University of Michigan*

**Hua Wang**
*National Institutes of Health*

# 2013 ASCI/AAP Joint Meeting Travel Award Recipients

**Feras Akbik**
*Yale University*

**Joseph L. Alge**
*Medical University of South Carolina*

**Neal Amin**
*Salk Institute*

**Vafa Bayat**
*Baylor College of Medicine*

**Lauren K. Brady**
*University of Pennsylvania*

**Ryan A. Denu**
*University of Wisconsin-Madison*

**Matthew L. Hedberg**
*University of Pittsburgh*

**Annie L. Hsieh**
*Johns Hopkins University*

**Tiffany Y. Hsu**
*Baylor College of Medicine*

**Rajan Jain**
*University of Pennsylvania*

**Jiyeon S. Kim**
*University of Pennsylvania*

**Leo Y. Luo**
*Broad Institute of Harvard and MIT*

**Nicholas O. Markham**
*Vanderbilt University*

**Kyle W. McCracken**
*University of Cincinnati/Children's Hospital*

**Brian D. Muegge**
*Washington University School of Medicine*

**Pankaj Pal**
*Washington University in St. Louis*

**Warren W. Pan**
*University of Michigan*

**Tyler P. Rasmussen**
*University of Iowa*

**Cecinio C. Ronquillo**
*John A. Moran Eye Center, University of Utah*

**Marc S. Sherman**
*Washington University in St. Louis*

**Jane W. Symington**
*Washington University in St. Louis*

**Maria C. Trissal**
*Washington University in St. Louis*

**Christine L. Tung**
*University of California San Diego*

**Samuel E. Vaughn**
*Cincinnati Children's Hospital and Medical Center*

**Yanjia J. Zhang**
*Harvard School of Public Health*

# 2013 APSA Annual Meeting Travel Award Recipients

**Christopher O. Audu**
*Dartmouth*

**Kristen A. Batich**
*Duke University*

**Sonali J. Bracken**
*University of Connecticut Health Center*

**Andres Chang**
*University of Kentucky*

**Irene Chernova**
*University of Pennsylvania*

**Stephen M. Chrzanowski**
*University of Florida*

**Dania Daye**
*Perelman School of Medicine at the University of Pennsylvania*

**Adam C. Diehl**
*Johns Hopkins University*

**Sarah E. Greene**
*Washington University in St. Louis*

**Brittany L. Gregory**
*University of Pennsylvania*

**Emily N. Guhl**
*University of Chicago*

**Bianca N. Islam**
*Georgia Health Sciences University*

**Stephanie R. Jackson**
*Saint Louis University School of Medicine*

**InYoung Kim**
*University of Chicago*

**Katherine L. Knorr**
*Mayo Clinic*

**Samuel D. Quaynor**
*Georgia Health Sciences University*

**Amy J. Reid**
*University of Texas Medical School at Houston*

**Michael J. Ripple**
*Louisiana State University Health Sciences Center*

**Casey S. Seldon**
*Georgia State University*

**Lulu Sun**
*Washington University in St. Louis*

**Josephine W. Thinwa**
*University of Texas Health Science Center San Antonio*

**Ting-Lin Yang**
*University of Pennsylvania*

**Wan R. Yang**
*Johns Hopkins University*

**Tresa E. Zacharias**
*University of Texas Southwestern Medical Center*

# 2013 ASCI Council Young Physician-Scientist Awards

The ASCI Council is pleased to recognize the recipients of its inaugural 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 27, 11:45 a.m. to 1:30 p.m.) to find out more about their work.

**Edward M. Behrens, M.D. (Poster: ASCI-1)**
*The Children's Hospital of Philadelphia*

**Kathrin Maria Bernt, M.D. (Poster: ASCI-2)**
*Children's Hospital Colorado/University of Colorado Denver*

**Maneesh Bhargava, M.D. (Poster: ASCI-3)**
*University of Minnesota*

**John M. Brehm, M.D. (Poster: ASCI-4)**
*Children's Hospital of Pittsburgh of UPMC / University of Pittsburgh*

**Carolyn S. Calfee, M.D., M.A.S. (Poster: ASCI-26)**
*University of California, San Francisco*

**Philip A. Chan, M.D., M.S. (Poster: ASCI-5)**
*Brown University*

**Scott P. Commins, M.D., Ph.D. (Poster: ASCI-6)**
*University of Virginia Health System*

**Edward Vincent Faustino, M.D. (Poster: ASCI-7)**
*Yale University School of Medicine*

**Alexander G. Fiks, M.D., M.S.C.E. (Poster: ASCI-8)**
*The Children's Hospital of Philadelphia*

**Brian Barkley Graham, M.D. (Poster: ASCI-9)**
*University of Colorado Denver*

**J. Anthony Graves, Ph.D., M.D. (Poster: ASCI-10)**
*Children's Hospital of Pittsburgh of UPMC*

**Steven K. Huang, M.D. (Poster: ASCI-11)**
*University of Michigan*

**Ania Magdalena Jastreboff, M.D., Ph.D. (Poster: ASCI-12)**
*Yale University School of Medicine*

**Qing Li, M.D., Ph.D. (Poster: ASCI-13)**
*University of Michigan*

**Jill Lamanna Maron, M.D., M.P.H. (Poster: ASCI-14)**
*Floating Hospital for Children at Tufts Medical Center*

**Tobias A. Neff, M.D. (Poster: ASCI-15)**
*Children's Hospital Colorado/University of Colorado Denver*

**Shetal H. Padia, M.D. (Poster: ASCI-16)**
*University of Virginia Health System*

**Matthew T. Rondina, M.D. (Poster: ASCI-17)**
*University of Utah*

**Lauren Hachmann Sansing, M.D. (Poster: ASCI-18)**
*University of Connecticut Health Center*

**Carla Rose Scanzello, M.D., Ph.D. (Poster: ASCI-19)**
*Rush University Medical Center*

**Jennifer Lynn Sherr, M.D., Ph.D. (Poster: ASCI-20)**
*Yale University School of Medicine*

**Neal J. Sondheimer M.D., Ph.D. (Poster: ASCI-21)**
*The Children's Hospital of Philadelphia*

**Jason Zachariah Stoller, M.D. (Poster: ASCI-22)**
*The Children's Hospital of Philadelphia*

**Andrew W. Tai, M.D., Ph.D. (Poster: ASCI-23)**
*University of Michigan*

**Dawn Marie Wetzel, M.D., Ph.D. (Poster: ASCI-24)**
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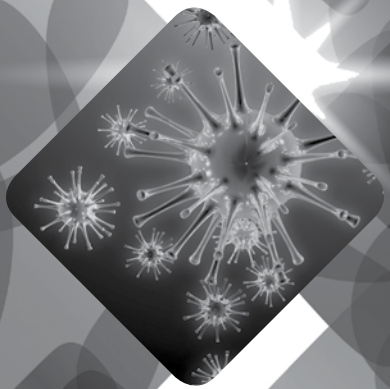
# Abstracts for Oral Presentations and Poster Session



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# Oral Presentations

## 1

### Role of FRS2 and FGF/FGFR Autocrine Signaling in the Proliferation of Ovarian Cancer Cells

***LY Luo*[1,2], HW Cheung[3], WC Hahn[1,4,5]**

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We have recently found FRS2 (fibroblast growth factor receptor substrate 2) to be essential in ovarian cancer through a genome-wide RNAi screen in over two hundred cancer cell lines. In addition, FRS2 is focally amplified in 15% of the primary serous ovarian tumors characterized by The Cancer Genome Atlas (TCGA) project, and expression level corresponds to the level of amplification. FRS2 encodes for an adaptor protein that mediates signal transduction downstream of fibroblast growth factor receptor (FGFR) upon activation by FGF ligands. We observed mutual exclusivity of FRS2 amplifications with FGFR amplifications or mutations in ovarian serous carcinoma. FRS2 has been shown to be recruited and phosphorylated by FGFR upon ligand binding and activates both phosphatidyl inositol (PI)-3 kinase and Ras/ERK pathways. Interestingly, we also observed that fibroblast growth factor 18 (FGF18) is frequently overexpressed in most ovarian cancer cells, but not other cancer types. Therefore, we hypothesize that the amplification of FRS2 and overexpression of its upstream signaling pathway promote proliferation and survival of ovarian cancer cells. We characterized the function and mechanism of FRS2 gene in ovarian cancer. Specifically, FRS2 is required for the survival of ovarian cancer cells that carry amplification of FRS2. Silencing FRS2 expression with independent short hairpin RNA (shRNA) caused increase anti-proliferative effect in FRS2-amplified ovarian cancer cell lines than non-amplified cell lines. Knockdown of FRS2 in amplified ovarian cancer cells caused a decrease in phospho-ERK level but not phospho-AKT level. This suggests the anti-proliferative effect is partially mediated by the MAPK signaling pathway. This is confirmed by an increase in phosphor-ERK Overexpression of FRS2 in 293T and IOSE cells. The data suggest a functional role of FRS2 amplification in cells that harbor such genetic alteration. Anchorage-independent growth assays are currently underway to identify its transforming potential. Together, these data will reveal the mechanistic roles of this oncogenic signaling and thus may credential a novel, promising target for ovarian cancer therapy.

## 2

### Heat Shock Protein 70 Demonstrates IL-10 Mediated Immune Modulation in Experimental Colitis

***I Kim*, W Wang, M Musch, E Chang, B Jabri**

*Committee on Immunology, Department of Gastroenterology, University of Chicago, Chicago, IL*

Heat shock protein 70 (Hsp70) is an inducible molecular chaperone whose expression is constituitive and robust in the large intestine, an area richly populated by gut microflora and other immune stimulators. Injection of recombinant Hsp70 in mice induces T cells that produce interleukin-10 (IL-10), but the molecular mechanism remains elusive. IL-10 producing regulatory-type 1 T cells (Tr1) are enriched in the intestines and play a critical role in intestinal immune homeostasis and in the prevention of inflammatory bowel disease (IBD). However, the mechanism by which Tr1 are generated *in vivo* is unknown. Previously, our laboratory showed that TLR6, which heterodimerizes with TLR2, confers immune-suppression by generating tolerogenic dendritic cells (DC) that further leads to the differentiation of Tr1 cells. Thus, we investigated whether Hsp70 induces Tr1 by the same mechanism. Indeed, Hsp70 promotes IL-10 producing tolerogenic DCs in a TLR2/6-dependent manner, which subsequently leads to Tr1 differentiation. Moreover, we found that like other known TLR2/6 ligands, Hsp70 also appears to be lipid-modified (palmitoylated). Interestingly, the significance of Hsp70 lipid-modification may be two-fold: 1) the lipid chains are presumably required for specific TLR2/6 receptor targeting, and 2) lipid modification is necessary for targeting to the surface for secretion. Hsp70 has traditionally been regarded as a strict intracellular molecule due to its lack of a leader peptide. Thus, the demonstration that Hsp70 is extracellularly exported created a paradigm shift in the study of these proteins. In particular, whether extracellular Hsp70 (eHsp70) exerts immune-modulatory or –stimulatory functions upon release into the extracellular milieu had remained a controversy. Not only does this study demonstrate a novel immune-modulatory cytokine function of a protein previously known to have functions restricted to the intracellular space, but also elucidates how a protein lacking a leader peptide may be exported. Finally, we showed that immune modulation via Hsp70 selectively expressed in the intestinal epithelium confers protection against experimental colitis. Medical interventions for IBD using recombinant IL-10 has shown limited effect. Instead, augmenting the induction of endogenous IL-10 seems more promising. Here, we present Hsp70 as a protein with amazing versatility, playing vital roles as a molecular chaperone to now an immune-modulating cytokine with a therapeutic potential.

# Oral Presentations

## 3

### T-bet Dictates CD8+ T Cell Tolerance Versus Immunity Following Antigen Recognition

***SR Jackson,*** **JY Yuan, MB Elliott, JM Meyer, RM Teague**
*Molecular Microbiology and Immunology, Saint Louis University School of Medicine, St. Louis, MO*

Adoptive T cell immunotherapy strives to cure cancer in human patients by transfer of tumor-reactive CD8+ T cells, but induction of T cell tolerance within the patient remains a major obstacle to achieving the anticipated clinical benefits of this approach. Efforts to overcome tolerance for improved immunotherapy have been hampered because the T cell intrinsic pathways that regulate whether transferred T cells are directed toward a tolerant versus an immune cell fate have not been defined. Using a murine model of CD8+ T cell tolerance, we have shown that adoptively transferred T cells that engage tumor antigen in a tolerizing context undergo multiple rounds of division, but fail to produce effector molecules and are progressively deleted without providing anti-tumor immunity. We defined the gene expression profiles for these tumor-reactive T cells after antigen encounter under tolerizing or immunizing conditions, and established that the T-box transcription factor, T-bet, which directs the effector differentiation of CD8+ T cells, fails to be induced within the tolerizing environment, but is induced in the immunizing environment. As a strategy to overcome this defect, T cells transferred into the tolerizing environment were vaccinated with attenuated *Listeria monocytogenes* (*Lm*). Restored T-bet in *Lm*-vaccinated T cells corresponded with their acquisition of effector function within the tolerizing environment. More importantly, *Lm* vaccination of leukemia-bearing hosts receiving adoptive T cell immunotherapy provided a significant survival benefit, with 40% achieving complete remission. To establish if T-bet was required for the rescue of CD8+ T cell tolerance during immunotherapy, we generated mice in which T-bet is deleted in tumor-reactive T cells. Utilizing these T cells, we demonstrated that T-bet was essential for rescue of effector cytokine production and cytolytic activity within the tolerizing (but not the immunizing) environment. Furthermore, immunotherapy with T cells lacking T-bet failed to improve the survival of leukemia-bearing hosts following vaccination. We are now performing studies using T cells with constitutive T-bet (CD2-T-bet) to determine if T-bet expression is sufficient to overcome tolerance and provide improved adoptive immunotherapy even in the absence of vaccination. Collectively, our data implicate T-bet as a cell-intrinsic regulator of effector responses by tumor-reactive CD8+ T cells during adoptive immunotherapy. These results have profound implications for improving T cell-based therapies for cancer patients and expand our understanding of the basic cell fate decision between tolerance and immunity.

## 4

### Systemic Type I interferons Indirectly Promote Epithelial Proliferation and Turnover

***L Sun*** **[1,2], DJ Morales[3], AC Barger, DJ Lenschow[4], TS Stappenbeck[2]**
*[1]MD-PhD Program, Departments of [2]Pathology and Immunology, [3]Molecular Microbiology, and [4]Medicine, Washington University in St. Louis School of Medicine, St. Louis, MO*

Epithelial barriers that undergo constant shedding and renewal are an important first-line immune defense against infection. However, the energy cost of perpetual turnover is high. Therefore, set points must be established that balance effective defense and metabolic efficiency. We hypothesized that such set points can be adjusted by cytokines of the innate immune system. Using a mouse model deficient in *Irgm1*, we uncovered a novel process involving Type I interferons (IFNs) that regulates epithelial turnover. Irgm1 is required for host defense against intracellular protozoan and bacterial pathogens. We investigated whether Irgm1 is also necessary for defense against viral infection. Surprisingly, *Irgm1*$^{-/-}$ mice were resistant to influenza A infection at doses lethal to wild-type (WT) mice. We discovered that Type I IFN levels were elevated in the lungs and serum of healthy, uninfected *Irgm1*$^{-/-}$ mice compared to WT mice. Since Type I IFNs are integral innate anti-viral cytokines, the augmented Type I IFNs in uninfected *Irgm1*$^{-/-}$ mice likely provided baseline protection against flu. This finding leads to the question of why circulating Type I IFNs are not always at increased levels to prevent viral infection. We therefore examined the effects of systemically persistent elevated Type I IFN levels on organ morphology and function. We found that the intestinal epithelium of *Irgm1*$^{-/-}$ mice was hyperproliferative, with augmented turnover, but without signs of infection or inflammation. Epithelial proliferation was also heightened in kidney, liver, pancreas and salivary gland, but not in lung, muscle and thyroid. The elevated Type I IFNs were responsible for the increased epithelial proliferation, as we were able to reverse the hyperproliferation phenotype by crossing *Irgm1*$^{-/-}$ mice with mice deficient in the Type I IFN receptor (Ifnar). Furthermore, induction of Type I IFNs in wild-type but not *Ifnar*$^{-/-}$ mice could also increase epithelial proliferation. Notably, *Irgm1*$^{-/-}$ mice that lacked Ifnar expression only in the intestinal epithelium still retained intestinal epithelial hyperproliferation. This result suggested that Type I IFNs act indirectly through an intermediate cell type to promote proliferation of the epithelium. Overall, these findings show novel negative regulation of basal Type I IFN production by Irgm1, and suggest that Type I IFNs can indirectly modulate the set point for epithelial proliferation and turnover. This function may contribute to IFN-mediated anti-viral defense.

# Oral Presentations

## 5
## Decreased Puberty and Fertility Development in NELF KO Mice Due to Impaired GnRH Neuron Migration

***S. D. Quaynor,*** **L.P. Chorich, R. S. Cameron, L. C. Layman**
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Normal puberty and reproduction require the proper development and function of the hypothalamic-pituitary-gonadal axis controlled by gonadotropin-releasing hormone (GnRH) neurons. GnRH neurons develop in the olfactory placode region and migrate into the brain, where they project their processes to the median eminence. Lack of migration of these neurons results in diseases such as Idiopathic Hypogonadotropic Hypogonadism (IHH) and Kallmann syndrome (KS). Patients with IHH/KS present with absent puberty, low gonadotropins and sex steroids in addition to anosmia in KS. Nasal embryonic luteinizing hormone releasing factor (NELF) is an important protein that has been identified to contribute to GnRH neuron migration, and mutations of the gene have been demonstrated in IHH/KS patients. We previously confirmed *Nelf*'s role in GnRH neuron migration using immortalized mice GnRH cell lines; and to date all the studies regarding *Nelf* have been done using immortalized neuronal cells and zebrafish models. To extend these studies into mammals, we generated homozygous Nelf knockout (KO) mice to further understand the phenotypic effect with regard to GnRH neuron function in the whole animal. We hypothesized that *Nelf -/-* mice will have impaired pubertal development and compromised fertility. To assess puberty of *Nelf* KO animals, we measured hormone panels (LH, FSH, testosterone, and estradiol) and determined vaginal opening and estrus onset. To determine the effect of *Nelf* KO upon fertility, we measured estrus cycles, and determined the number of days between litters and the litter sizes of both homozygous males and homozygous females when they were mated with wild type animals. Finally, we confirmed the defects in GnRH neuron migration and cell number through the performance of immunohistochemistry on serial brain slices using GnRH antibody. Our preliminary results indicate that female *Nelf -/-* mice have delayed vaginal opening by five days compared with wild type mice. In addition, both male and female *Nelf -/-* mice have impaired fertility as manifested by a decreased litter size and increased time between litters. Preliminary evidence suggests that GnRH neuronal migration distance is decreased in *Nelf* KO mice compared to wild type. These data support our clinical findings of human NELF mutations and show that NELF plays an important role in both normal pubertal development and fertility.

# Poster Abstracts

## 1

## Angiotensin II Promotes Mitochondrial Oxidative Stress in Atria

***APH Ackell**, O Koval*, O Jaffer*, M Joiner*, ME Anderson***
**Department of Internal Medicine, University of Iowa, Iowa City, IA*

**Background:** Angiotensin II (Ang II) causes heart failure, atrial fibrosis and sinus node dysfunction in mice. Mitochondrial reactive oxygen species (ROS) are amplified following Ang II, leading to the activation of several downstream redox-sensitive pathways important for cardiac hypertrophy, apoptosis and arrhythmias. The aim of this study was to investigate the role of Ang II in promoting mitochondrial ROS in mouse atria. We found that Ang II infusion leads to the oxidation of mitochondrial $Ca^{2+}$/calmodulin-dependent protein kinase II (CaMKII) into a constitutively active form (ox-CaMKII) within atrial mitochondria. Activated mitochondrial CaMKII signals to the mitochondrial $Ca^{2+}$ uniporter (MCU) leading to increased mitochondrial $Ca^{2+}$ uptake, increased mitochondrial stress and cell death while mitochondrial CaMKII inhibition is cardioprotective. We hypothesized that Ang II increases mitochondrial ROS in atria through mitochondrial CaMKII activation and increased mitochondrial $Ca^{2+}$ entry. **Methods:** We measured mitochondrial ROS production in mouse atrial myocytes using mitoSOX Red staining and in atrial mitochondria using a lucigenin superoxide quantification assay. Cells and mitochondria were isolated from mice which received Ang II, Ang II + mitoTEMPO (a mitochondrial-targeted antioxidant) or vehicle infusions for three weeks. To determine the oxidation status of mitochondrial CaMKII in these animals, we quantified the protein levels of ox-CaMKII and total CaMKII in mitochondrial and cytosolic fractions of atrial lysates. Mitochondrial $Ca^{2+}$ conductance was assessed using patch clamp electrophysiology. We generated patch-clamp viable mitoplasts (mitochondria lacking outer membranes) from mice treated with Ang II, Ang II + mitoTEMPO or vehicle in order to record MCU currents. **Results:** Atrial myocytes from animals treated with Ang II have increased mitochondrial ROS levels and increased expression of ox-CaMKII. The ROS elevations and increase in ox-CaMKII were rescued by mitoTEMPO co-treatment. Ang II increases MCU-mediated mitochondrial $Ca^{2+}$ conductance and this can be blunted with mitoTEMPO infusion. **Discussion:** We conclude Ang II increases mitochondrial ROS levels and mitochondrial $Ca^{2+}$ entry through the MCU and that this key stress response may be under the control of activated mitochondrial CaMKII signaling. As future work, we seek to inhibit mitochondrial CaMKII and MCU conductance to determine if this lowers mitochondrial ROS levels and if mitochondrial CaMKII activation and MCU conductance represent future targets for preventing or treating atrial disease.

## 2

## Defective Generation of B-cell Memory Following Vaccination in Patients With Sickle Cell Disease

***AJ Adami,* SM Szczepanek, D Lewis, SJ Bracken, ER Secor, ER Jellison, RS Thrall, B Andemariam**
*University of Connecticut Health Center, Farmington, CT*

**Introduction**: Sickle cell disease (SCD) is an immunological enigma. Despite arising from a mutation in an erythrocyte-specific protein, increasing evidence suggests systemic immunologic changes and inflammation are wrought by the disease. Early mortality associated with infection is a hallmark of SCD, necessitating early and frequent vaccination. However, the efficacy of vaccines is not well studied in this population. Recent evidence suggests that vaccine efficacy is poor in SCD, and anecdotal evidence from clinicians suggests that SCD patients mount sufficient initial antigen-specific antibody responses following vaccination only to see antibody titers drop to undetectable levels 1-2 years post-vaccination (behavior not observed in the general population). To explore this, we conducted a clinical translational study to evaluate the B-cell memory response of patients with SCD to routine vaccination against influenza virus. **Methods**: Blood samples from healthy adult controls and SCD patients were collected before and 4-6 weeks after vaccination, and peripheral blood mononuclear cells were analyzed by flow cytometry for expression of surface lineage markers. **Results**: The relative percentages of CD19+ B-cells were similar between control subjects and SCD patients before vaccination, but CD19+ B-cells nearly doubled in the SCD patients after vaccination while almost no change was observed in controls. A concurrent increase in absolute B-cell number was also observed in SCD patients after vaccination, while control subjects saw a contraction. However, analysis of CD27+ memory B-cells revealed almost no increase in the SCD patients versus a significant increase (approximately 2-fold) in the controls. This suggests that SCD patients generate naïve, but few to no memory, B-cells after vaccination and may explain declining antibody titers observed by clinicians. Further analysis of B-cells from this study revealed a significantly higher proportion of FcRL4+ exhausted B-cells at baseline in SCD patients over controls. FcRL4 expression inhibits BCR and increases TLR signaling, acting as an adaptive to innate switch to modulate B-cell responsiveness. Interestingly, high expression of FcRL4 by B-cells is associated with reduced memory B-cell generation in chronic HIV or malaria infection. **Conclusions**: Our findings indicate that patients with SCD undergo an expansion of naïve but not memory B-cells after vaccination, reducing vaccine protection. We hypothesize that the mechanism may involve FcRL4-mediated dampening of BCR signaling due to chronic inflammation. Funding: Lea Center for Hematologic Disorders, Neag Cancer Center, UConn Health Center

# Poster Abstracts

## 3

## Nogo Receptor 1 Titrates Anatomical Plasticity of Adult Brain

***Feras V. Akbik,*** **Sarah M. Bhagat, Pujan R. Patel, William B.J. Cafferty and Stephen M. Strittmatter**
*Cellular Neuroscience, Neurodegeneration and Repair Program, Departments of Neurology and Neurobiology, Yale University School of Medicine, New Haven, CT*

In completed studies, we have shown that blockade of myelin inhibitor action by Nogo Receptor 1 (NgR1) decoy or by gene deletion partially overcomes myelin inhibition of axonal growth *in vitro*, and supports a significant degree of axon plasticity, sprouting and regeneration after spinal cord injury. Moreover, ocular dominance plasticity in visual cortex is maintained at critical period levels far into adulthood when NgR1 is absent. However, NgR1's role in the anatomical stability of synapses of the adult brain is unknown. We used time-lapse, transcranial two-photon microscopy to measure the turnover of dendritic spines and axonal varicosities in the somatosensory cortex of mice lacking NgR1. Experience can alter anatomical connectivity in the brain, but such plasticity is strongly suppressed in adulthood. Through adolescence, the neuronal anatomy and plasticity in the brains of NgR1 null mice are indistinguishable from control. Unlike wild-type mice, NgR1 mutants do not undergo age-dependent stabilization of synaptic turnover in the somatosensory cortex after age 26 days. In fact, conditional deletion of NgR1 in one-year old mice reactivates 1-month old levels of anatomical plasticity. Suppression of anatomical dynamics by NgR1 is cell autonomous and is phenocopied by deletion of Nogo-A ligand. Whisker removal deprives the somatosensory cortex of experience-dependent input and reduces dendritic spine turnover in the brains of adult NgR1 null mice to control levels, while acute exposure to an enriched environment increases dendritic spine dynamics in control mice to the level of NgR1 mutant mice housed in a standard environment. Thus, NgR1 determines the low set point for synaptic turnover in adult cerebral cortex and increases the threshold to anatomically imprint experience. Functionally NgR1 mutants show enhanced learning in motor training and fear conditioning, two behavioral paradigms that are linked to cortical spine turnover. NgR1 is an adult-onset, reversible brake for anatomical plasticity that determines the low-set point for synaptic turnover in adult cerebral cortex and increases the threshold to anatomically imprint experience with functional relevance. Reactivating juvenile rates of cortical plasticity by NgR1 loss-of-function therefore represents a novel therapeutic mechanism to maximize recovery and rehabilitation after neurological injury.

## 4

## Urinary Renin Improves Prediction of Worsening Acute Kidney Injury

***Joseph L. Alge*[1] and John M. Arthur[1,2]**
*[1]Medical University of South Carolina, Charleston, SC; [2]Ralph H. Johnson VA Medical Center, Charleston, SC*

**Background:** Acute kidney injury (AKI) is a common, life threatening condition associated with an increased risk of adverse outcomes. The risk of adverse outcomes is proportional to the maximum stage of AKI achieved. Thus, once the diagnosis of AKI has been established, there is a need to reliably estimate a patient's risk of progression to more severe AKI (worsening AKI). Elevated urinary angiotensinogen/creatinine ratio (uAnCR), a prognostic biomarker of AKI, is an early predictor of worsening AKI, but a high percentage of patients who progress to more severe AKI are classified as intermediate risk by uAnCR. In the current study we tested the hypothesis that other biomarkers of AKI could improve prediction of worsening AKI in patients predicted to be intermediate risk by uAnCR. **Methods:** Urine was obtained from 59 post-cardiac surgery patients with established AKI, 42 of whom were classified as intermediate risk for worsening AKI based upon their uAnCR values. Urinary NGAL, KIM-1, Cystatin-C (Cys-C), IL-18, and renin were measured by ELISA and corrected for urine creatinine to account for biological variability in urine dilution. The area under the ROC curve (AUC) was used to evaluate the ability of each biomarker to predict worsening of AKI after the time of sample collection (defined as progression to a higher AKIN stage) among the intermediate risk subset. **Results:** Thirteen patients were classified as low risk and 4 were classified as high risk using uAnCR, of which 2 and 3 patients, respectively, experienced worsening of AKI. Twelve of 42 patients in the intermediate risk group met the outcome. Urinary renin was significantly elevated in these patients compared to those whose AKI did not worsen (median values of 1.19 vs 0.59 ng renin / mg urine creatinine, respectively; p=0.02). Renin predicted worsening of AKI (AUC=0.74 95% CI 0.57 - 0.90), and had a sensitivity and specificity of 75% and 60% at its optimal cut-off (0.68 ng/mg). The established AKI biomarkers NGAL, KIM-1, Cys-C, and IL-18 did not predict worsening of AKI in the intermediate risk group. **Conclusions:** Elevated urinary renin is associated with more severe AKI and predicts worsening of AKI in patients classified as intermediate risk based on their uAnCR values. Renin could be used in combination with uAnCR to more accurately identify AKI patients who are at high risk of adverse outcomes.

## 6

## Effects of Combined Retinoic Acid and Valproic Acid Towards the Neurogenesis of ESC-derived Neural Stem Cells

***Radhian Amandito*, Berry Juliandi**
*Nara Institute of Science and Technology (Takayama, Ikoma, Nara, Japan)*

**Introduction**: Neural stem cells (NSC) have a myriad of potential uses, such as the treatment for spinal cord injury. Even though neural stem cells have the ability to self-renew and generate various neural cell types, to use them as treatments the control of differentiation is vital. Therefore, research on the mechanisms of NSC differentiation must be done. Both retinoic acid (RA) and valproic acid (VPA) have been proven to be involved in neurogenesis in mice model; VPA as an HDAC inhibitor to induce neuronal differentiation and RA which enhances histone H3 acetylation to induce astrocyte differentiation of NSC. The objective of this research is to find the most suitable substance and/or combination of substances to be used in neuronal differentiation of ESC-derived NSCs. **Materials and Methods:** NS cells were derived first from mouse ES cells. DMEM Ham's F-12 along with Vitamin B27, Penicillin Streptomycin Fungicide, EGF, and BFGF was used. Passaging was done using Hank's Buffered Salt Solution (HBSS). For experiment cells were moved to a

four-well dish with N2 medium and FGF; control, RA, RA+FBS, RA+VPA. Immunocytostaining was done with antibodies for Tuj1, GFAP, and nestin, and then observed under fluorescent microscope. Cell count was done to determine differentiation of cells in each dish. **Results**: In treatment of the VPA and RA, there is indication that neurogenesis enhanced compared to the cells that was only treated with RA and the control dish. The enhancement is mostly shown in the Tuj1 immunofluorescence where it is more abundant. However the number of astrocyte differentiation was also increased in the combination of RA and VPA treatment. **Discussion:** This indicates that there is no specific fate preference from the treatment. It does however indicate that during the incubation period there was an increase in cell proliferation and differentiation of NSCs when treated with a combination of VPA and RA.

## 7

## Alternative Processing of Slit Transcripts Yields Insights Into Motor Neuron-Specific RNA Regulatory Mechanisms

***ND Amin,*** **W Gifford, S Driscoll, S Pfaff**
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**Background:** Motor neurons are located in the ventral spinal cord and control all the muscles in the human body. In diseases such as spinal muscular atrophy and amyotrophic lateral sclerosis, these neurons degenerate, leaving patients progressively weaker until they are completely unable to move. Many genes have been linked to these diseases, and they are believed to play a role in RNA splicing, transport, and turnover. However, little is known about the molecular mechanisms of RNA processing that set apart motor neurons from other cell types, or why motor neurons selectively degenerate when these ubiquitously expressed genes are disrupted in humans. My work seeks to understand RNA processing events that may be specific to motor neurons, including microRNA generation, alternative splicing and alternative polyadenylation. **Results:** I have identified microRNA-218 (miR-218) as specifically expressed by all classes of spinal motor neurons, beginning with their generation and continuing into post-natal stages. MicroRNA sequencing of motor neurons and non-motor spinal neurons identified miR-218 as the most differentially regulated microRNA in motor neurons, suggesting it has a highly specific role in motor neuron transcript regulation. Predictive analysis has identified Robo1, Reelin, and Retinoic Acid Receptor-alpha as putative targets of miR-218, suggesting a role in regulating axon guidance, neuronal migration, and motor neuron-specification. Interestingly, this microRNA is encoded at two sites within the genome - within the introns of the canonical axon guidance molecules Slit2 and Slit3. We performed RNA-sequencing on motor neurons to study the RNA processing mechanisms of these two miR-218 loci within their Slit "host" genes. This analysis identified novel promoters for both of these genes, as well as a predominance of novel alternatively spliced and alternatively polyadenylated Slit3 transcripts. These alternative splice- and polyadenylation-site are located adjacent to the pre-miR-218 hairpin, which is highly suggestive of a functional relationship between microRNA biogenesis and co-transcriptional RNA processing from Slit3 transcripts in motor neurons. **Future work:** I will continue to study the molecular mechanisms that underlie miR-218 biogenesis and the alternative processing of Slit transcripts, as well as the *in vivo* function of this microRNA through the generation of miR-218 knockout mice.

## 8

## Malignancy & Risk for Suprapopliteal DVT After Endovenous Laser Ablation Therapy

***Rozana H. Asfour,*** **Philip G. Gomez, Michael J. Malinowski, Jonathan Unkart, and Bernadette Aulivola**
*Department of Vascular Surgery, Loyola University Medical Center, Maywood, IL*

**Objective:** Deep venous thrombosis (DVT) is a known potential complication after greater saphenous vein (GSV) laser ablation. Given the morbidity associated with DVT, we sought to identify factors that may increase the risk of developing this complication after GSV endovenous laser therapy (EVLT). **Methods:** A retrospective medical record review was performed on all patients who underwent GSV laser ablation at a single institution from January 1, 2008 to May 31, 2011. Data collected included demographics (age & sex), procedural details (concomitant varicose vein excision), comorbidities (history of malignancy, diabetes, hypertension, hyperlipidemia, DVT, superficial thrombophlebitis), peri-operative medications (aspirin, Plavix, warfarin), and pre-operative ultrasound characteristics. Univariate logistic regression was performed to assess significant independent variables. A multivariate logistic regression with backwards elimination model was used to identify significant variables. **Results:** Review identified 233 limbs undergoing GSV laser ablation during the study period. All patients underwent pre and post-operative venous duplex of the affected extremity. Twenty-three were diagnosed with acute DVT with 7.3% of those having suprapopliteal DVT on postoperative duplex. Of variables assessed, age (OR 1.03, 95% CI: 1.00-1.07, p=0.010) and a history of malignancy was found to significantly increase the risk of post-operative DVT with an odds ratio of 4.47 (95% CI: 1.44-13.95, p=0.003) in the final multivariate logistic regression model. DVT rate was 6/18 (33.3%) in patients with and 17/179 (7.9%) in patients without a history of malignancy. The majority of the femoral vein DVTs were Type II EHIT types across all groups, however, the patients with malignancy history had a significantly higher prevalence of suprapopliteal DVTs compared to non-malignancy patients presenting with supra, mixed, and infrapopliteal thrombosis. **Conclusions:** Patients with a history of malignancy have an increased risk of developing clinically significant DVT after EVLT, conferring potential benefits for directed treatment modalities for DVT prophylaxis in the perioperative period.

# Poster Abstracts

## 9

### Kruppel-like Factor 2 Protects Against Ischemic Stroke by Regulating Endothelial Blood Brain Barrier Function

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**Background:** During an ischemic stroke normal brain endothelial function is perturbed resulting in blood brain barrier (BBB) breakdown with subsequent infiltration of activated inflammatory blood cells, ultimately leading to neuronal cell death. Kruppel-like factor 2 (KLF2) is regulated by flow, is highly expressed in vascular endothelial cells, and serves as a key molecular switch regulating endothelial function and promoting vascular health. **Purpose:** To determine the role of KLF2 in cerebrovascular function and the pathogenesis of ischemic stroke. **Methods and Results:** To examine the specific role of KLF2 in the brain and cerebrovascular disease, we performed ischemic strokes and conducted BBB assays in KLF2 deficient ($K2^{-/-}$), KLF2 overexpressing ($K2^{tg}$), and control mice. $K2^{-/-}$ mice exhibited larger strokes in the transient middle cerebral artery occlusion model (95% greater stroke volume vs. control; n=10-20/group; *P*=0.001) and significant impairment in BBB function after stereotactic TNFα injection as assessed by *in vivo* real time quantitative PET analysis and evans blue dye (EBD) assays (86% greater EBD permeability vs. control; n=3/group; *P*=0.002). In contrast, $K2^{tg}$ mice were protected against ischemic stroke (49% smaller stroke volume vs. control; n=8-9/group, *P*=0.004) and demonstrated less impairment in BBB function after stereotactic TNFα injection (30% less EBD permeability vs. control; n=3/group; *P*=0.003). In concordance, gain and loss of function studies in primary brain microvascular ECs using transwell assays revealed KLF2 to be BBB protective (32% less permeability after oxygen glucose deprivation in adenoviral KLF2 overexpression vs. control; *P*=0.005 and 107% greater permeability at baseline in siRNA mediated KLF2 knockdown vs. control; *P*<0.0005). Mechanistically, KLF2 was demonstrated to regulate the critical BBB tight junction factor occludin, both *in vitro* (7.0±0.3 fold expression in adenoviral KLF2 overexpression vs. control; *P*<0.0001 and 0.51±0.01 fold expression in siRNA mediated KLF2 knockdown vs. control; *P*=0.01) and *in vivo* (0.56±0.11 fold expression in $K2^{-/-}$ vs. control; n=4-7/group; *P*=0.01 and 2.5±0.5 fold expression in $K2^{tg}$ vs. control; n=3-4/group; *P*<0.05). **Conclusions:** These data are first to identify KLF2 as a key regulator of the BBB and a novel neuroprotective factor in ischemic stroke.

## 10

### Engineering a Soluble Parathyroid Hormone Receptor

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**Background:** We designed and characterized a soluble mimic of the parathyroid hormone (PTH) receptor (PTH1R) that incorporates the N-terminus and third extracellular loop of PTH1R, important for ligand binding. The engineered receptor (PTH1R-NE3) was conceived to enable easy production and the use of standard biochemical and biophysical assays for the screening of competitive antagonists of PTH. Antagonists to PTH1R, a membrane protein belonging to the class B G-protein coupled receptor family, may provide new therapeutic options for calcium metabolism diseases like hyperparathyroidism malignancy. We used fluorescence polarization, photoaffinity labeling, and NMR experiments to show that PTH1R-NE3 is folded, thermodynamically stable and selectively binds PTH. We also identified a small molecule that competes with PTH in our PTH1R-NE3-based fluorescence polarization assay. These results demonstrate that PTH1R-NE3 recapitulates hormonal binding to the wild-type receptor and can serve as a tool for identification of competitive antagonists.

## 12

### Biochemical Characterization of Proteasomal Catalytic Activity in Three Solid Tumor Cell Lines

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The remarkable therapeutic effect of the proteasome inhibitor Bortezomib, for the treatment of both solid and hematological cancers, has opened a promising direction of research for the discovery of a novel generation of chemotherapeutics which target the Ubiquitin-Proteasome System (UPS). UPS-directed chemotherapeutics result in higher tissue selectivity and lower side effects because the UPS can interfere with cancer progression via specific molecular pathways. It is critical to determine the baseline activity of the proteasome in cancer cells, as well as the effect of proteasome inhibitors, because proteasome composition, catalytic activity, and subcellular localization are cancer- and tissue-specific. We utilize an iodixanol ultra-centrifugation method to analyze the catalytic activities of native proteasome complexes with intact associated protein partners which can guide with the discovery of new proteasome-dependent signaling pathways and proteasome associated protein partners to serve as therapeutic targets in cancer. In this study we identify differences in the subcellular distribution, catalytic activity, and inhibitor sensitivity of proteasomes in colon, breast, and pancreatic cancer cell lines. Ultimately, our results will serve as a valuable guideline for investigators developing chemotherapeutics for solid tumor cancers.

# Poster Abstracts

## 13

## Identifying the Rold of TULA-2, a Novel Tyrosine Phosphatase in Bone Remodeling

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**Background:** Hematopoietic stem cells (HSC) differentiate into osteoclasts when the cell surface receptors, c-FMS and RANK, are activated by their ligands M-CSF and RANKL respectively. In addition to c-FMS and RANK stimulation, another calcium-mediated, co-stimulatory pathway must also be activated to ensure proper osteoclast differentiation. This pathway is activated by two immunoreceptors, OSCAR and TREM-2, that associate with two transmembrane adapter proteins, FcRγ and DAP12 respectively, which contain immunotyrosine activation motifs (ITAM). ITAMs are cytoplasmic domains that contain tyrosine residues that become phosphorylated upon stimulation of their respective immunoreceptors. These phosphotyrosines act as docking sites for the tyrosine kinase, Syk. Once recruited, Syk autophosphorylates and acts on downstream targets such as PLCγ2 to mediate osteoclast differentiation and function. The reversible phosphorylation of Syk is therefore, necessary to regulate osteoclast formation and function. Recently, a novel tyrosine phosphatase, T-cell Ubiquitin ligand -2 (TULA-2) has demonstrated the ability to dephosphorylate specific phosphotyrosine residues on Syk in various systems and has shown an increased specificity to dephosphorylate tyrosine 346. TULA-2 is a member of the TULA family of proteins, TULA and TULA-2. In spite of a significant homology and similar domain organization between TULA and TULA-2, only TULA-2 has significant phosphatase activity. Furthermore, whereas TULA is expressed only in lymphocytes, TULA-2 is expressed in most tissues albeit a higher level of expression is seen in cells of hematopoietic origin. The goal of our project is to determine how TULA-2 regulates skeletal remodeling. We hypothesize that TULA-2 negatively regulates osteoclast function by dephosphorylating Syk and attenuating the downstream signaling cascade.

## 14

## Rapamycin Rescue of Tauopathy is Associated with Increased Accumulation of Immature Autophagosomes — Evincing a Gridlock

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Tauopathies are a group of neurodegenerative diseases characterized by intracellular aggregates containing the microtubule-associated protein tau. Autophagy activation reduces toxicity of aggregation-prone proteins, but whether autophagy plays a central role in tauopathies remains largely a matter of conjecture. In a well-characterized *Drosophila* model, human tau induces accumulation of autophagic intermediates with a preponderance of large double membrane-bounded vacuole; these are incompletely acidified and contain a mixture of digested and undigested material, likely representing a less functional autophagic entity. We term these vacuoles giant autophagic bodies (GAB). Lowering basal autophagy in the presence of tau reduces GAB, whereas increasing autophagy with rapamycin treatment in the presence of tau produces a decrease in mature autolysosomes but an increase in GAB. However, GAB formation does not directly contribute to generation of the toxic phenotype of human tau, which is suppressed by rapamycin. Taken together, these data suggest that activation of autophagy in tauopathy impedes overall autophagic flux. And despite their immaturity, the ensuing accumulation of large autophagic intermediates may actually serve a neuroprotective role, analogous to a proposed role of large polyglutamine aggregates in Huntington's disease.

## 15

## Kinetochore-microtubules Mediate Radiation-Induced Genome Damage

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The exquisite sensitivity of mitotic cancer cells to ionizing radiation underlies the rationale for fractionated radiation therapy. Nonetheless, the mechanism of this cell cycle-dependent vulnerability is unknown. Here we show that ionizing radiation selectively increases the stability of microtubule attachments to chromosomes at kinetochores, eliciting a dose-dependent surge of kinetochore-microtubule attachment errors during chromosome segregation. These errors, manifested by lagging chromosomes in anaphase, generate long-term aneuploidy, a preponderance of micronuclei, and chromosome pulverization. Destabilizing, or temporarily abolishing, microtubule attachments to chromosomes leads to reduction of these defects, substantial increase in the viability of irradiated mitotic cells, and radiation resistance in orthotopically transplanted human glioblastoma multiforme tumors. Alternatively, pharmacologically increasing kinetochore-microtubule attachment errors potentiates radiation-induced genome damage. Thus, kinetochore-microtubules represent prominent cellular targets of ionizing radiation that lead to widespread genome damage beyond direct DNA breaks and they offer additional means to sensitize tumors to radiation therapy. Finally, to emphasize the clinical relevance of these findings, we show that increased rates of lagging chromosomes in rectal adenocarcinomas substantiates an enhanced response to chemo-radiation therapy thereby supporting

the role of whole-chromosome mis-segregation in mitigating radiation-induced damage.

## 16

## Prioritizing Genetic Testing in Patients with Kallmann Syndrome Using Clinical Phenotypes

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**Background:** The complexity of genetic testing in Kallmann Syndrome (KS) is growing and costly. Thus, it is important to leverage the clinical evaluations of KS patients to prioritize genetic screening. The objective of this study is to determine which phenotypes in KS subjects have implications for specific gene mutations. **Material & Methods:** We studied 219 KS probands who were selected from a cohort of 568 probands participating in a genetic study at the Reproductive endocrine Unit of the MGH: 151 with known rare sequence variants (RSV) in 8 genes known to cause KS (KAL1, NELF, CHD7, HS6ST1, FGF8/FGFR1 or PROK2/PROKR2) and 68 KS subjects who remain RSV-negative for all 8 genes. Also, reproductive and non-reproductive phenotypic features within each genetic group were derived from: clinical charts of patients, notes from referring physicians, and patient questionnaires. **Results:** Male KS subjects with KAL1 RSVs displayed the most severe reproductive phenotype with testicular volumes (TV) at presentation of 1.5 ± 0.1 ml. vs. 3.7 ± 0.3 mL, $p<0.05$ vs. all non-KAL1. In both sexes, synkinesia was enriched in patients with KAL1 RSVs compared to KAL1 negative probands (43% vs. 12%; $p<0.05$). Likewise, dental agenesis and digital bone abnormalities were enriched in patients with RSV in the FGF8/FGFR1 signaling pathway compared to all other gene groups combined (39% vs. 4% and 23% vs. 0%; $p<0.05$, respectively). Hearing loss marked the probands with CHD7 RSVs (40% vs.13% in non-CHD7 probands; $p<0.05$). Renal agenesis and cleft lip/palate did not emerge as statistically significant phenotypic predictors. **Conclusion:** Certain clinical features in men and women are highly associated with genetic causes of KS. Synkinesia (KAL1), dental agenesis (FGF8/FGFR1), digital bony abnormalities (FGF8/FGFR1), and hearing loss (CHD7) can be useful for prioritizing genetic screening.

## 18

## Variations in Response to Newly Developed Oral Anticoagulants in Normal and Liver Disease Patients

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**Background:** With cardiovascular and liver diseases ranked as leading causes of death in the U.S. these pathologies will coincide in some patients, thus complicating anticoagulant selection and dosing in management of thrombotic disorders in this population. The introduction of newer oral Xa inhibitors such as Rivaroxaban (Xarelto, Janssen Pharma and Bayer AG), and Apixaban (Eliquis, Pfizer Inc. and Bristol-Myers Squibb), and IIa inhibitor Dabigatran (Pradaxa, Boehringer Ingelheim Pharma) has added a new dimension in management of thrombotic disorders. Although these drugs were claimed to have certain advantages over conventional anticoagulants such as no monitoring requirements, fixed dosage and decreased side effects, more recent data has pointed to safety issues and differential responses. This study was designed to evaluate how fixed dosing affects anticoagulant responses of both healthy and liver disease patients on an individual and population based level. **Methods:** De-identified samples with suspected liver disease by high icteric index (n=50) were collected from clinical laboratories and control samples (n=30) were obtained from healthy volunteers. Each plasma sample was supplemented with individual anticoagulants (Dabigatran, Apixaban, Rivaroxaban) or saline at a concentration of 250 ng/mL. Prothrombin time (PT) and activated partial thromboplastin time (aPTT) were measured using Innovin and Platelin reagents, respectively. Thrombin generation studies were performed using a Thromboplastin-triggered fluorogenic substrate method. **Results:** Scatterplots were constructed to show variability of normal and liver disease individuals with data compiled as mean, standard deviation, and percent change of clotting time. In PT assays, percent increase of clotting time ranged from 2.5-6% in liver disease patients, whereas the normal showed an average 2% increase. All anticoagulants increased aPTT clotting time, with Dabigatran showing the greatest elevation in liver disease (42.2±26.3sec) compared to normal (36.5±8.8 sec). The average aPTT increase for normal (3.5-14%) was less than for liver disease samples (6-15%). Wide scatter was noted in the thrombin generation assay among both groups with much higher average thrombin generation in liver disease patients. **Conclusions:** These results underscore not only the population based variations, but also the differences in the anticoagulant and thrombin generation inhibitory responses with new anticoagulants. Therefore, monitoring and individual dosing may be required to optimize the therapeutic outcome with the newer anticoagulants.

## 19

## Memory Responses Govern Dendritic Cell Migration to Vaccine-Site Draining Lymph Nodes with Resultant Enhanced Anti-Tumor Efficacy

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**Background**: Dendritic cells (DCs) are a promising entity for the realization of successful immunotherapy against cancers, but one inherent limitation is that generally <5% of intradermally administered DCs actually reach vaccine-site draining lymph nodes (VDLNs). In a randomized clinical trial, we found that DC migration to VDLNs via conditioning with tetanus-diphtheria (Td) toxoid was significantly enhanced compared to controls, and efficiency of DC migration strongly correlated with survival in

brain tumor patients. Preclinical data reveal that an intact memory response to Td antigen is responsible for driving the trafficking of injected DCs, elucidating a novel role for the memory response in governing DC homing to VDLNs. **Methods**: A preclinical model comprising DCs derived from GFP transgenic mice was employed to explore DC migration in the context of Td conditioning versus a non-inflammatory control. The effects of enhanced DC migration on tumor-specific immune responses and tumor growth were evaluated. **Results**: In accordance with the clinical trial, Td conditioning given before DC vaccination significantly enhanced migration of DCs to VDLNs (*P<0.001*). DC migration was only enhanced in mice with an intact memory response to Td (*P<0.01)*. Specifically, memory T cells to Td played a critical role in mediating DC migration, as the adoptive transfer of Td-specific T cells conferred enhanced DC migration in naïve mice (*P<0.01)*. Td conditioning given before a DC vaccine provided a sustained elevation in OVA-specific T cells over time compared to controls (*P<0.001)*. Furthermore, Td conditioning dramatically suppressed tumor growth in a subcutaneous model of the highly aggressive B16-OVA-expressing melanoma tumor (*P<0.001)*. **Conclusion**: Td conditioning dramatically enhanced DC migration and tumor antigen-specific immune responses in a preclinical model corroborating effects of a Td skin preparation on DC migration observed within our clinical trial. Identifying inflammatory mediators elicited by the memory response that govern migration of DC vaccines offers the potential to monitor these mediators as predictors of cellular vaccine efficacy in patients with highly aggressive cancers.

## 20

## Effects of Chronic Amphetamine Treatment on Cocaine-Induced Facilitation of Intracranial Self-Stimulation in Rats

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Amphetamine is a monoamine releaser and candidate agonist medication for cocaine dependence. Chronic amphetamine treatment decreases cocaine self-administration by rats, nonhuman primates and humans. This study tested the hypothesis that chronic amphetamine would decrease abuse-related facilitation of intracranial self-stimulation (ICSS) by cocaine in rats. Male Sprague-Dawley rats were equipped with electrodes targeting the medial forebrain bundle and trained to respond under a fixed-ratio 1 schedule for 0.5 sec trains of electrical stimulation. Stimulation intensity was individually determined in each rat, and frequency varied from 56-158 Hz in 0.05 log units during each session. Effects of 10 mg/kg cocaine on ICSS were determined before, during and after 14-day continuous infusion with saline (N=5) or amphetamine (0.32 mg/kg/hr, N=5). Saline treatment had no effect on ICSS, and cocaine produced comparable facilitation of ICSS before, during and after saline treatment. Amphetamine facilitated ICSS throughout the 14-day treatment, and termination of amphetamine decreased ICSS below pre-treatment baseline. Cocaine facilitated ICSS before and after amphetamine, but during amphetamine treatment, it did not facilitate ICSS above levels produced by amphetamine alone. These findings suggest that chronic amphetamine decreases abuse-related stimulus effects of cocaine. Funded by R01 DA026946.

## 21

## Complications and Outcomes of Jehovah's Witness Patients Following Total Joint Arthroplasty

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**Introduction:** Total joint arthroplasty (TJA) is associated with significant blood loss. Blood transfusion may be necessary for some patients. Recent literature suggests an increase in negative outcomes associated with transfusion of allogenic blood products. However, refusal to accept blood transfusion may also be detrimental to patient outcomes and lead to increased complications. Jehovah's Witness' refuse all blood transfusion due to religious beliefs. The purpose of this study is to evaluate the outcome of patients undergoing TJA who refused blood transfusion. **Methods:** We retrospectively reviewed all patients undergoing TJA at our facility during the past 10 years (May 2001 to December 2011). We identified 24 Jehovah's Witness patients who refused any transfusion. We compared this group to matched controls. Length of stay, post-operative Hgb levels, transfusion requirements and complications were analyzed. **Results:** Compared to matched controls, Jehovah's Witness patients had an increased length of stay in the hospital (4.92 days vs 3.9 days). They also had a higher incidence of post-operative anemia (mean post-op Hgb 10.1 vs 12.2), though no transfusions were given. There was no difference in the incidence of major complication. Outcome scores did not demonstrate a difference. **Discussion** and **Conclusion:** Jehovah's Witness patients who refuse blood transfusion had a longer length of stay than controls. They also had higher incidence of postoperative anemia. However, there was not an increase in the incidence of complications and overall their outcomes were no different than controls. It is our position that Jehovah's Witnesses, and thereby all patients who refuse transfusion, may undergo elective TJA without increased risk of major complication or a negative impact on outcome. Furthermore, our experience with Jehovah's Witness patients has improved our management of all patients allowing us to be more selective with transfusion of blood products.

## 22

## Mutations in the Mitochondrial Methionyl-tRNA Synthetase Cause a Neurodegenerative Phenotype in Flies and a Recessive Ataxia (ARSAL) in Humans

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Studies in fruit flies have provided significant insights into our understanding of the mechanisms by which neurodegeneration occurs in certain mutants. In a forward genetic screen in our lab, we identified missense mutations in the *Aats-met* gene, coding for the mitochondrial methionyl-tRNA synthetase, the ortholog of human *MARS2*. These proteins are required during protein synthesis in mitochondria. The fly mutations have been successfully rescued by expression of *Drosophila* and human cDNAs. We also

confirmed using a Flag tag that the protein is mitochondrially localized. Homozygous mutant clones produced with the eyFLP/FRT technique reveal a gradual loss of synaptic transmission over the course of four weeks as gauged by the progressive loss of depolarization in electroretinograms, suggesting a progressive degeneration. Additionally, Transmission Electron Microscopy of aged *Aats-met* mutant eye tissue display a disorganized and severely altered morphology of photoreceptors, with greater mitochondrial mass and lipid droplets in both the retinal and laminar layers. Interestingly, the mutant phenotypes are milder at 18°C, allowing for the generation of transheterozygous escapers. These escapers exhibit neurodegenerative phenotypes, reduced lifespan, and flight muscle degeneration. We subsequently found that the mitochondria exhibit defects in Complex I and an increase in Reactive Oxygen Species levels. In addition, these mitochondria exhibit an Unfolded Protein Response, suggestive of protein misfolding. We also noted that mutant tissues were smaller and found this to be due to decreased cell proliferation secondary to ROS. Additionally, antioxidants improve survival to adulthood, the degeneration phenotype, and overall eye appearance and size. Finally, we found that mutations in the human *MARS2* locus are responsible for the neurological disease ARSAL (Autosomal Recessive Spastic Ataxia with Associated Leukoencephalopathy), which had been mapped to this region. Analysis of 60 patients show that they exhibit different combinations of three rearrangements in the *MARS2* gene, identified by Copy Number Variation analysis. In addition, these patients have reduced levels of the MARS2 protein as well as mitochondrial translation defects, and patient cells have higher ROS levels and reduced cell proliferation rates. Thus, our *Drosophila* study of *Aats-met* will provide insight into the progression and pathology of this and related diseases.

## 23

## How are Pain Behaviors and Pain Processing Altered in Severe Alzheimer's Disease?

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**Background:** Though studies of pain in Alzheimer's disease (AD) indicate that the disease alters pain related affect and cognition, no studies have examined how clinically observable patient pain responses relate to changes in cerebral pain processing. Further, knowledge of how pain is affected by severe Alzheimer's disease (sAD) is limited. We predicted that, compared to healthy seniors (HS), sAD patients would have reduced resting state functional connectivity between brain regions responsible for pain affect associated with reduced behavioral and autonomic responses to acute pain stimuli. **Methods:** Five pressure intensities (1-5kg/cm$^2$) were applied to the forearms of subjects (four applications each | 11 HS / 17 sAD subjects). 50s rest periods separated each 5s stimulus. Autonomic (HR) and behavioral responses (scored using the Pain Assessment in Advanced Dementia scale) were recorded. HS self-reported pain with the Faces Pain Scale. Seed correlation analysis determined resting-state fMRI functional connectivity among 16 pain-related regions of interest after standard pre-processing (6 HS / 4 sAD subjects). Pair-wise r-coefficients were Z-transformed for group comparisons (two-tailed T-test). Results: Each increase in pressure yielded significant increases in pain ratings by HS ($F=34.1$, $p<0.001$). While sAD subjects had reduced HR responses, except for the highest pressure ($F=13.97$, $p<0.001$), they were more behaviorally responsive to pressure ($F=11.995$, $p<0.001$). sAD subjects, compared to HS, had increased insular connectivity to the hypothalamus. However, sAD had reduced functional connectivity between secondary somatosensory cortex to medial and dorsolateral prefrontal cortex. **Conclusions:** The hypothalamus, which is affected by AD pathology, helps mediate the affective motivational dimension of pain by coordinating threat defense behaviors and autonomic responses. Increased functional connectivity from other medial pain regions (e.g., insula) may indicate a predisposition for increased behavioral responsiveness and dysfunctional autonomic responses to both innocuous and painful stimuli. Meanwhile, greater connectivity in HS between medial and dorsolateral prefrontal regions to secondary somatosensory cortex could indicate more efficient gating of pain stimuli. Determining how functional changes in pain-related brain structures manifests in observable pain behaviors of sAD patients has potential for improving patient pain assessment and quality of life.

## 25

## Transient Inactivation of the Subthalamic Nucleus Decreases the Essential Value of Cocaine

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Economic essential value of a drug has been shown to correlate with several clinical measures of addiction in humans. In rats essential value has been shown to predict cue-induced reinstatement of methamphetamine and cocaine seeking, an animal model of relapse. The current study determined the role of the rat brain subthalamic nucleus (STN) in driving the essential value of cocaine. Consumption of cocaine was measured at 11 ascending cocaine prices (lever responses/mg cocaine) in a single 110-min session. Rats were pretreated with bilateral microinjections (0.3 µL) into STN of either vehicle (artificial cerebrospinal fluid) or the $GABA_A$ receptor agonist muscimol (0.2 mM) prior to testing in a within-subjects crossover design. Muscimol pretreatment significantly attenuated the essential value of cocaine compared to vehicle or injections of muscimol immediately dorsal to STN. In contrast, muscimol treatment did not alter cocaine consumption when the price of cocaine remained low throughout the session (e.g., FR1 schedule), indicating that STN inactivation results in price-dependent changes in cocaine consumption. Given the clinical promise of economic measures of drug use, these results support a possible clinical utility of STN inactivation in treating cocaine abuse.

# Poster Abstracts

## 26

## The Effects of *Trypanosoma* Cruzi Infection and Vaccination on Priming *T. cruzi*-specific CD4 T Cells

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**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. We have generated transgenic (Tg) mice with a monoclonal $CD4^+$ T cell population specific for the TSaa57-74 peptide found to be important in the induction of *T. cruzi* immunity. However, we have identified defects in $CD4^+$ T cell responses after *T. cruzi* infection as compared to TS vaccination. Therefore, we sought to compare the Tg TS-specific $CD4^+$ T cell population induced by infection versus vaccination through RNA microarrays. **Methods:** Tg TSaa57-74-specific $CD4^+$ T cells were purified from naïve transgenic mice and activated both *in vitro* and *in vivo*. In vitro, $CD4^+$ T cells were stimulated with dendritic cells alone, DCs pulsed with TSaa57-74 peptide, DCs infected with *T. cruzi*, and DCs pulsed with TSaa57-74 and infected with *T. cruzi*. After 24 hours, surface markers of activation were studied by flow cytometry and RNA was harvested for microarray analysis. We also primed the Tg TSaa57-74-specific $CD4^+$ T cells *in vivo* by adoptively transferring Tg $CD4^+$ T cells into naïve BALB/c mice and activating them by *T. cruzi* infection versus TS vaccination. At various time points post immunization, the Tg $CD4^+$ T cells were purified and RNA extracted. RNA was analyzed by microarray with Illumina MouseWG-6v.20 Beadchips and Partek software. Fold changes were calculated and unadjusted ANOVA was used to define significance ($p<0.05$). **Results:** Following *in vitro* stimulations, surface expression of CD69 and CD25 were similar among Tg T cells stimulated with TSaa57-74 alone and TSaa57-74 plus *T. cruzi* infection. However the microarray revealed a subset of genes that were significantly altered between these two groups. Infection of the antigen presenting cells resulted in an increase in the expression of genes involved in apoptotic pathways, among others. Following *in vivo* activation, proliferation, as measured by CFSE dilution, was similar among Tg T cells stimulated with TS vaccination and *T. cruzi* infection. The microarray revealed genes differentially expressed in response to *T. cruzi* infection. Further analysis of these distinct transcriptomes is ongoing. **Conclusions:** These *in vitro* and *in vivo* studies using our novel transgenic *T. cruzi*-specific $CD4^+$ T cells suggest that *T. cruzi* infection and TS vaccination differentially activate $CD4^+$ T cells. This may explain the defects in $CD4^+$ T cells observed following *T. cruzi* infection and can perhaps be manipulated to improve $CD4^+$ T cell responses.

## 27

## Birth Stories: The Social Context of Decision-Making in Childbirth Management

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**Background:** The aim of this project is to examine the social context of childbirth management in Southeast Michigan. In medical anthropology discourse, birth is viewed as being a cultural process—a result of not only physiologic events, but also deeply-engrained values and beliefs from the setting in which they take place. This concept is demonstrated by the widely varied approach to birth management across the globe. Medicine is also shaped by more than just empirical data. Regardless, American biomedicine is often mistakenly viewed as a purely objective science, rather than a powerful force of dominant Western ideologies. As such, birth, acting as a point of intersection between biology, American medicine, and social variables, serves as a mirror of community dynamics relating to race, socioeconomic status, access to care, and social relations between healthcare providers, their patients, and the medical institution. Through observation and in-person recorded interviews, this research study will elicit patient and provider perspectives on desired birth settings, attendants, and interventions. Additionally, we will compare perspectives between urban and suburban participants, contrasting the distinct geographical settings in order to construct a clearer understanding of the influence of economic disparities, racial/ethnic diversity, resource allocation, and health insurance status on the decision-making process. In using an anthropological approach, this research adds a unique and valuable perspective on childbirth and the role of both community and biomedical culture in these clinical interactions

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## Analysis of the Risk Profile of Patients Under 40 Years for Pulmonary Thromboembolism in the Intensive Care Unit

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**Objective:** Analyze the epidemiological profile of patients under 40 years with a diagnosis of pulmonary embolism (PE) in an intensive care unit (ICU) in Brasília-Brazil. **Methods:** It was made an interview survey with patients or family members, data of 102 patients admitted to the ICU of the Hospital Santa Lucia, Brasília-DF. It is an observational study design for patients under age 40 years and risk profiles associated to PE. **Results:** Of the patients, 27 were older than 40 years, with 59, 3% female. The risk factors with the highest prevalence were: recent surgery (48.1%), sedentary (51.9%) and contraceptive use (40.7%). The presence of hypertension was 14.8% and 3.7% diabetes mellitus. Smokers accounted for 7.4% and 3.7% former smokers. Restrictions locomotion was observed in 25.9%, 3.7% with a recent trauma, 14.8% were obese, 7.4% thrombophilic, 7.4% had signs of venous insufficiency, 11.1% had a family history of PE/deep vein thromobosis (DVT), 18.5% had a history of PE/DVT, 7.4% were in using of aspirin/anticoagulants. No individual had other factors, among those studied. The presence of earlier event of PE/DVT ($p = 0.002$) was significantly to the development of a new episode. **Conclusion:** In young patients the presence of previous event PTE/DVT is suggestive of a new episode of PE. It is also important recent surgery, with almost half of the individuals evolving with TEP, demonstrating importance in the development/enhancement of prevention for this disease.

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## ERK2-Regulated TIMP-1 Induces Hyperproliferation in K-*Ras*$^{G12D}$ Transformed Pancreatic Ductal Cells

***Gregory P. Botta*[1, 2], Maximilian Reichert[2], Mauricio J. Reginato[1], Steffen Heeg[2], Anil K. Rustgi[2, 4], Peter I. Lelkes[3, 4]**

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K-*Ras*$^{G12D}$ mutated pancreatic ductal adenocarcinoma (PDAC) conceals a desmoplastic reaction composed of deregulated, proliferating cells embedded in an abnormal extracellular matrix (ECM). Our previous observations imply that inhibiting the MAPK-ERK2 kinase signal pathway reverses an MMP-1 specific invasive phenotype. Here we investigated the specific genes downstream of MAPK-ERK2 responsible for the hyperproliferative abilities of human and murine primary ductal epithelial cells (PDECs) within an ECM. Human PDECs harboring the PDAC-common p53, Rb/p16$^{INK4a}$, and K-*Ras*$^{G12D}$ mutations had significant increases in DNA synthesis and total cell proliferation over control cells; an observation readily reversed following small-molecule inhibition or lentiviral silencing of ERK2. Human microarray analysis of PDECs in 3D culture determined that a unique, MAPK-influenced gene signature is expressed downstream of K-*Ras*$^{G12D}$. Unbiased hierarchical analysis of K- *Ras*$^{G12D}$ upregulated genes that are downregulated by MAPK inhibition filtered tissue inhibitor of matrix metalloproteinase (TIMP)-1 as a gene whose expression was expressively pathway specific. Indeed, K-*Ras*$^{G12D}$ mutated mice that have developed PDAC exhibit increased TIMP-1 RNA and protein abundance within the pancreatic compartment compared with wild type littermate controls. Analyses of both 3D, *in vitro* human K- *Ras*$^{G12D}$ PDECs and public annotated human pancreatic datasets correlatively indicate TIMP-1 RNA and supernatant/serum protein increases. While silencing TIMP-1 did not significantly effect PDEC proliferation, exogenous addition of human recombinant TIMP-1 sufficiently increased proliferation, but only in transformed K-*Ras*$^{G12D}$ PDECs in 3D. Overall, TIMP-1 is an upregulated gene product and sufficient proliferative inducer of K-*Ras*$^{G12D}$ mutated PDECs via the ERK2 signaling pathway.

## 30

## Resolution of Pulmonary Function Abnormalities Following Chronic Exposure to House Dust Mite Antigen in a Murine Model of Asthma

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**Background:** House dust mite (HDM) is the most common cause of asthma worldwide due to its widespread immunogenic properties. Our laboratory has previously established that chronic administration of ovalbumin in a murine model of allergic airway disease (AAD) results in development of *immunological tolerance* and resolution of disease parameters, including airway hyperreactivity. However, it is not known whether the increased immunogenicity of HDM impairs development of tolerance, thus explaining its global prevalence. In this study, we aim to determine whether chronic administration of HDM can result in development of immunological tolerance in a murine model of AAD. **Methods:** C57BL/6 mice were challenged intranasally with 25 µg HDM extract 5 days/week for two (acute), five (sub-acute), or eleven (chronic) weeks to induce AAD. Control animals received equal volumes of PBS. Mice were anesthetized and mechanically ventilated for assessment of pulmonary function using the forced oscillation technique (SCIREQ flexiVent system). **Results:** Mice receiving HDM for 2 weeks demonstrated a significant increase in total respiratory system resistance over control animals that peaked after 5 weeks of exposure to antigen, indicating development of airway hyperreactivity. In addition, acute administration of HDM resulted in a significant decrease in lung compliance that worsened at sub-acute stages of disease. Following chronic administration of HDM, total respiratory system resistance and compliance improved to levels observed in control animals. These results correlated with a significant decrease in eosinophils in the broncheoalveolar lavage fluid and an increase in Foxp3+ T regulatory cells (Tregs) in the hilar lymph node at this time point. **Conclusion:** Our data indicates that chronic exposure to HDM results in development of immunological tolerance, as marked by resolution of airway hyperreactivity, improvements in total lung compliance, and alterations in the local immunological profile that skew in favor of Tregs. *This work was funded by:* NIH/AI R01 HL-43573 (RST), T32AI007080 (SJB).

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## Regulation of Alternative Splicing in Human Cells Exposed to Ionizing Radiation

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Radiation exposure is common in diagnostic and therapeutic settings. Individuals differ in sensitivity to radiation. The mechanisms underlying individual variation in response to radiation remain poorly understood. In this project, we are studying the mechanistic basis of cellular and gene expression response to radiation with the ultimate goal of improving ways to predict and influence therapeutic outcomes in radiotherapies. We carried out RNA-sequencing of cultured B-cells from normal individuals before and after exposure to ionizing radiation. The results revealed an unexpected layer of complexity; we found a set of genes that change patterns of splicing following radiation exposure. Some of these genes were previously not known to play a role in radiation-response, as many of these complex changes could not be detected by measuring total gene expression with microarrays. These isoform changes have been validated at the mRNA and protein levels. The isoforms differ by expressed exons, transcription start sites and UTR lengths. Among these genes, the shorter isoforms were preferentially expressed in irradiated cells ($P_c < 0.01$). These shorter gene isoforms differ in transcript stability and encode proteins with altered functional domains,

thus likely to have different molecular functions. One example is the histone methyltransferase, SUV420H1, whose shorter isoform maintains the catalytic domain but loses a region that mediates heterochromatin binding. Even though the short and long isoforms of this gene share some common targets, by RNA interference, we found several hundred genes whose expression levels are influenced by the short isoform. Many genes play roles in cell signaling and alternative splicing, such as an interferon alpha-inducible protein, *IFI6*, a mediator complex subunit, *MED17*, and a serine/threonine kinase involved in chromatin assembly, *TLK1* ($P < 0.01$). Thus, the differential expression of the short and long isoforms of *SUV420H1* affects genes that mediate chromatin dynamics, cell cycle progression and transcriptional regulation. In this presentation, I will show data on the roles of alternate splicing and chromatin modification in radiation response.

## 32

## Testing Cold Feet: The Roles of Ion Channels TRPM8 and TRPA1 in Cold Sensation

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Background: Pain is an important clinical problem that has required at least $99 billion in federal and state medical expenditures in 2008 alone. Recent studies have shown that pain is not adequately managed in a many patients, including postoperative patients and cancer patients. Cold pain is a particularly clinically relevant subset of pain, as broad populations including multiple sclerosis, chemotherapy, and stroke patients all experience types of cold hypersensitivity. Two ion channels that may be involved in aberrant cold sensation are the transient receptor potential (TRP) channels TRPM8 and TRPA1, which are believed to respond to cold stimuli. Despite intense study of these channels over the last ten years, there is still some controversy over the role of TRPA1 in cold pain due in part to limitations in the behavioral assays currently available to measure cold responses *in vivo*. To complement the assays currently in use, we have applied our new assay of cold response threshold, the cold plantar assay, to further define the roles of TRPM8 and TRPA1 in acute, inflammatory, and neuropathic cold responses. Supported by NINDS funds R01NS42595 and 1F31NS078852-01A1.

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## Preventing Vision Loss by Vascular Stabilization in Diabetic Retinopathy

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Proliferative diabetic retinopathy is the leading cause of blindness in young people and the most common cause of retinal neovascularization. Hyperglycemia induces endothelial damage to the retinal vessels creating hyperpermeability and ischemia, which leads to angiogenesis. Currently, no therapies exist for stabilizing the vasculature in diabetic retinopathy. Here, we show that a single intravitreous dose of adeno-associated virus serotype 2 encoding a stable, soluble form of angiopoietin-1 (AAV2.COMP-Ang1) ameliorated structural and functional hallmarks of diabetic retinopathy in the Ins2Akita mouse model of type 1 diabetes with sustained effects observed through six months. AAV2. COMP-Ang1 increased VE-cadherin expression and decreased VEGF-A expression in the Ins2Akita mouse retina. COMP-Ang1 preserved vascular network area comparable to non-diabetic control levels, despite persistent pericyte dropout, and returned vascular hyperpermeability to control levels. Furthermore, AAV2.COMP-Ang1 therapy prevented retinal thinning and ganglion cell layer dropout. Most importantly, diabetic mice treated with COMP-Ang1 retained visual acuity and electroretinographic response. Stabilizing the vasculature with AAV2.COMP-Ang1 prevented functional loss in the diabetic retina.

## 35

## Analysis of KAL1, FGFR1, GPR54 and NELF Mutations by Multiplex Ligation Dependent Probe Amplification (MLPA) in Male Patients With Idiopathic Hypogonadotropic Hypogonadism

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**Background:** Hypogonadotropic hypogonadism is the failure in production of gonadal hormones from lack of gonadotropin secretion. Here we aimed to determine the prevalence of KAL1, FGFR1, GPR54 and NELF mutations in patients diagnosed with hypogonadotropic hypogonadism. **Material** and **Methods:** 86 male patients with idiopathic hypogonadotropic hypogonadism (76 diagnosed with normosmic idiopathic hypogonadotropic hypogonadism (nIHH) and 10 with Kallmann syndrome) and 95 healthy controls were recruited in the study to investigate KAL1, FGFR1, GPR54 and NELF mutations, using multiplex ligation dependent probe amplification (MLPA). **Results:** From the 86 patients, 3 patients with Kallman syndrome had heterozygous deletions in exon 9 of the KAL1 gene (probe target sequence: 5941-L05940) and one of these patients had also a duplication in exon 11 of the same gene (probe target sequence: 4427-L03813). 1 patient with nIHH had a duplication in exons 14 and 18 of the FGFR1 gene (probe target sequences: 4440-L03826 and 4441-L03827 respectively). No deletions / duplications were identified in the GPR54 and NELF genes and no mutations were detected in the control subjects. **Conclusion:** To improve our understanding of this complex disorder, for a better genetic counseling and for directing therapy, defining the genetic basis of these disorders is essential.

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### Targeting Cancer's Sweet Tooth – Development of Novel Anti-Glycolytic Therapeutics to Exploit the Warburg Effect

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Many cancers have been shown to have altered central metabolism, consuming high rates of glucose and excreting large quantities of lactate, a phenomenon known as the Warburg effect. It has been elucidated in recent years that cancers' elevated expression of the insulin-independent glucose transporter GLUT-1, as well as of the glycolytic enzyme lactate dehydrogenase A (LDH-A), provide a molecular basis for cancers' dysfunctional metabolism. However, strategies exploiting these facets of cancer metabolism have yet to make an impact in the clinic. In order to target both cancers' upregulation of both GLUT-1 and LDH-A, we have developed a glucose-conjugated *N*-hydroxyindole class lactate dehydrogenase inhibitor. We have demonstrated that this compound enters cells via GLUT transporters, where it then reduces cancer cells' lactate production and leads to cancer cell death. Further work on this compound is in progress, including elucidating its metabolic and transcript profiles compared to other published LDH inhibitors, and determining its safety and efficacy in murine tumor models.

## 37

### Pattern of NAV1.1 Expression During Development

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Dravet Syndrome (DS) is a childhood disorder in which symptoms develop as the patient ages. These characteristics include seizures that are AED resistant, severe cognitive deficits and alterations in EEG. It has been observed that many DS patients, as well as patients with other neurological disorders, have a loss-of-function genetic mutation on the SCN1a gene that effects the coding for the voltage-gated sodium channel type I (Nav1.1). To better understand the age-dependent neurological consequences of Nav1.1 loss of function, we characterized its expression in rats at various ages ranging from birth early adulthood. Fluorescent and DAB histological staining techniques were used. We found that brain Nav1.1 expression increases with age in a spatial gradient where hindbrain structures express the channel first and neo cortical areas last. Such developmental pattern could be directly linked to the progression of seizure types and neurological impairments in Dravet syndrome, hypothesis that we will test in animal models of the disease. Knowing the timing of disease progression in specific brain structures will help in the design of therapeutic interventions targeting the functions they support.

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### Hearing Thresholds in Rats Following Chronic High-Dose Vicodin Exposure

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The acetaminophen/hydrocodone combination commonly known as Vicodin is one of the highest selling pharmaceuticals in the US. It is also a chronically abused drug and has been linked with sudden sensorineural hearing loss in a subset of patients taking high doses. Previous research used an in-vitro mouse model and showed that exposure to high levels of Vicodin caused cochlear hair cell death. We sought to examine the effects of chronic high doses of Vicodin on hearing thresholds using an *in-vivo* rat model. In this study, four groups of six rats were given daily doses of either hydrocodone, acetaminophen, hydrocodone/acetaminophen (Vicodin), or yogurt as a control for four months. On days 0, 30, 60, 90, and 120 an auditory brainstem response / (ABR) test was performed to measure hearing thresholds. We found that there was no significant hearing loss associated with any of the groups over the four month course of exposure. Our findings are in contrast to prior reports that suggested exposure to high concentrations of acetaminophen/hydrocodone played a causative role in sudden sensorineural hearing loss. These results may suggest that sudden sensorineural hearing loss observed in patients with a history of Vicodin abuse could be the result of a comorbidity.

## 39

### 2-Deoxy-D-Glucose Sensitizes Colorectal Cancer Cells to TRAIL-induced Apoptosis

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The need for new, targeted therapeutic strategies for cancer treatment is apparent. For example, colorectal cancer (CRC) is the third leading cause of cancer-related mortality and yet therapeutic options are limited for those ~50% of patients presenting with metastasis. TNF-Related Apoptosis Inducing Ligand (TRAIL) is an endogenous cytokine that induces apoptosis selectively in cancer cells. However, in Phase III clinical trials the 30 CRC patients enrolled were refractory to TRAIL regimens likely due to the several resistance mechanisms identified *in vitro*. Experimentally, resistance to TRAIL can be overcome using a number of agents that induce states of cellular stress. Therefore, we sought to determine if we could selectively induce a stressed state in cancer cells to overcome TRAIL resistance. The work of Dr. Otto Warburg elucidated the phenomenon that cancer cells and tumors tend to have increased glycolytic flux and reliance on glycolysis, as opposed to respiration, for energy relative to normal adjacent tissue. This Warburg Effect is utilized clinically for diagnostic and staging purposes of cancer using Fluorodeoxyglucose as a biomarker because the glucose analog cannot be metabolized and preferentially accumulates in many tumors. Similarly, we attempted to selectively enhance TRAIL susceptibility in cancer cells by using the glucose deprivation mimetic, 2-Deoxy-D-Glucose (2DG). Utilizing HT-29 and SW-620 TRAIL-resistant,

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CRC cell lines, we studied the effect of glycolysis inhibition by 2DG on their sensitivity to TRAIL. 2DG and TRAIL co-treatment resulted in exponential increase in cell death over time relative to controls. Cell death characterization revealed it to be caspase-dependent and due to the enhancement of extrinsic apoptotic signaling. Using a subcutaneous xenograft model in athymic mice, five consecutive days of 2DG+TRAIL treatment resulted in modest tumor regression relative to controls. To explain this phenomenon, mechanistic studies revealed enhanced expression levels of TRAIL cognate receptor, death receptor 5 (DR5) with no effect on death receptor 4 (DR4). The unique ability of mannose supplementation to abrogate this synergy, as opposed to other exogenous monosaccharides, suggests that the mechanism of TRAIL sensitization may be due to an effect of 2DG other than glycolysis inhibition. Further mechanistic elucidation may reveal new therapeutic strategies for the treatment of colorectal cancer with implications for other tumor types.

## 40

## Human Metapneumovirus uses Cell Surface Heparan Sulfate Proteoglycans as an Attachment Factor for Cell Entry in Immortalized Human Bronchial Epithelial Cells

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**Background:** The paramyxovirus human metapneumovirus (HMPV) is a cause of serious viral respiratory disease in infants, the elderly, and in immunocompromised patients. It is second in prevalence only to the closely related Respiratory Syncytial Virus. Despite its clinical significance, little is known about its entry pathway, complicating the search for antivirals. HMPV expresses 3 surface glycoproteins: the attachment protein G, a small hydrophobic protein SH, and the fusion protein F. **Methods:** To systematically dissect the key players in binding and entry, recombinant wild-type (WT) HMPV, and recombinant viruses lacking either the G protein (ΔG) or the G and SH glycoproteins (ΔGΔSH) were tested in a variety of cell types for viral binding and infectivity. **Results:** Two independent binding assays showed that the absence of glycosaminoglycans greatly impacted WT and mutant virus binding and consequently protected the Vero and CHO cells from viral infection. HMPV binding and infection was inhibited in cells not expressing heparan sulfate (HS) and also in cells treated with heparinases. Confocal imaging showed that HMPV particles colocalize with HSPGs present at the cell surface. Treatment with heparinases, however, did not have an effect on PIV5 infection indicating that binding to HS is specific for HMPV. These results were replicated in immortalized non-cancerous human bronchial epithelial cells (BEAS-2B), which has been used as a model for human respiratory epithelium. Furthermore, no significant differences in binding and infection were detected in the absence of the G and/or SH proteins, which indicate that binding of the HMPV F protein to heparan sulfate proteoglycans is the major interaction driving viral attachment. **Conclusion:** We demonstrated that the F protein is the major protein driving attachment of HMPV through interactions with cell surface HSPGs and that this interaction is important in a model of human respiratory epithelium. Disruption of the interactions between HMPV F and HPSGs could potentially serve as a viable antiviral strategy.

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## mTOR Inhibition Prevents All-trans Retinoic Acid-induced Gut-homing Phenotype in Naturally Occurring Treg but not in Conventional CD4+ T Cells

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**Background:** CD4+ FoxP3+ regulatory T cells (Treg) are a population of suppressor T cells that have shown promise in treating experimental autoimmune disease, allograft rejection, and graft-versus-host disease. We have shown that the vitamin A metabolite all-trans retinoic acid (ATRA) and the mTOR inhibitor rapamycin, two compounds which improve the generation of induced Treg, have differential effects on chemokine receptor expression, migration, and therapeutic efficacy of murine induced-Treg. In this study, we investigated the influence of all-trans retinoic acid and rapamycin on naturally-occurring Treg. **Methods:** Freshly-isolated C57BL/6 CD4+ CD25+ Treg or CD4+ CD25- naive T cells were cultured with ATRA, rapamycin, and/or TGF-b, and analyzed by flow cytometry. **Results:** In contrast to conventional T cells which uniformly upregulated the gut-homing chemokine receptor 9 (CCR9) in the presence of ATRA, we found that only about half of naturally-occurring Treg upregulated CCR9, with delayed kinetics. The bimodal distribution of CCR9+ versus CCR9- Treg subsets was not due to differences in the activation marker CD62L, extent of proliferation, or intensity levels of FoxP3. Furthermore, we found that the presence of the mTOR inhibitor rapamycin prevented the appearance of the CCR9+ fraction in naturally-occurring Treg, but did not interfere with ATRA-induced CCR9 upregulation in conventional T cells. This effect was specifically due to inhibition of mTORC2, as Rictor$^{-/-}$ Treg (which lack mTORC1) showed no diminishment of the CCR9+ fraction compared to wild type control. **Conclusions:** Compared to conventional T cells, naturally-occurring Treg are not as easily polarized to become gut-homing, and they are susceptible to rapamycin blockade of ATRA-induced CCR9 expression, revealing a novel difference between Treg and conventional T cell biology. These findings also provide a framework for which the migratory behavior of Treg versus conventional T cells can be differentially tailored.

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## Pulmonary Inflammation After Ethanol Exposure and Burn Injury is Attenuated in the Absence of IL-6

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Alcohol consumption leads to an exaggerated inflammatory response after burn injury. Elevated levels of interleukin-6 (IL-6) in patients are associated with increased morbidity and mortality after injury, and high systemic and pulmonary levels of IL-6 have been observed after the combined insult of ethanol

exposure and burn injury. To further investigate the role of IL-6 in the pulmonary inflammatory response, we examined leukocyte infiltration and cytokine and chemokine production in the lungs of wild-type and IL-6 knockout mice given vehicle or ethanol (1.12 g/kg) and subjected to a sham or 15% total body surface area burn injury. Levels of neutrophil infiltration and neutrophil chemoattractants were increased to a similar extent in wild-type and IL-6 knockout mice 24 hours after burn injury. When ethanol exposure preceded the burn injury however, a further increase of these inflammatory markers was seen only in the wild-type mice. Additionally, signal transducer and activator of transcription-3 (STAT3) phosphorylation did not increase in response to ethanol exposure in the IL-6 knockout mice, in contrast to their wild-type counterparts. Visual and imaging analysis of alveolar wall thickness supported these finding and similar results were obtained by blocking IL-6 with antibody. Taken together, our data suggest a causal relationship between IL-6 and the excessive pulmonary inflammation observed after the combined insult of ethanol and burn injury. (This work was supported by R01AA012034 (EJK), T32AA013527 (EJK), F32AA018068 (MDB), F31 AA019913 (AZ) an Illinois Excellence in Academic Medicine Grant, The Margaret A. Baima Endowment Fund for Alcohol Research, and the Dr. Ralph and Marian C. Falk Medical Research Trust.)

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## Challenge be *MET*: Structural and Cell Biological Characterization of MET Receptor Tyrosine Kinase

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**Background:** Receptor tyrosine kinase (RTK) represents a large family of evolutionarily conserved cell-surface receptors. Their activation by growth factors (GF) regulates fundamental cellular processes and disease progression such as in cancers. Inhibiting deregulated RTKs thus provides a powerful therapeutic approach in cancer treatment. The overall goal of my project is to elucidate the molecular basis of signal transduction by Hepatocyte Growth Factor (HGF) through its receptor MET receptor tyrosine kinase (MET RTK). In humans, activation of MET RTK on cell surface by HGF plays pivotal roles in embryonic development, morphogenesis, tissue repair, and when deregulated, progression of malignancy. Over-expression of MET, for instance, correlates with poor prognosis and contributes to metastasis of lung cancer, colorectal cancer, and glioblastoma, among others. Also, stromal cell-secreted HGF is capable of conferring tumor resistance to many existing kinase inhibitors, thus countering the effectiveness of our current treatment. Additionally, HGF/MET engages other surface signaling complexes implicated in cancer progression, such as Wnt-Frizzled, Semaphorin/Plexin, and VEGF/VEGFR pathways. Based on mounting clinical and basic research data, HGF/MET is emerging as a strongly desired target in oncology. Yet, despite their diverse roles in malignancy, the fundamental mechanisms of MET receptor recognition and activation by HGF remain poorly understood. Over the past years, we have solved the crystal structure of the MET extracellular region hypothesized to mediate receptor dimerization and consequent activation. Correlating structure with function, we are employing cell-based assays to test specific residues required for receptor regulation. The information should not only refine our broader knowledge of the diverse signaling mechanisms of the RTK superfamily, but also facilitate therapeutic development against cancers. Future works will be dedicated to elucidate how HGF and MET specifically recognize one another to initiate cancer signaling pathways.

## 44

## Essential Role of γδ T Cell-Derived IL-17A in Protection Against *Clostridium difficile* Infection

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*Clostridium difficile* infection (CDI) is the most prevalent hospital-acquired infection, resulting in gastrointestinal disease with symptoms ranging from mild diarrhea to sepsis and death. CDI is most strongly associated with antibiotics use and represents a significant risk to hospitalized patients. The recent emergence of antibiotics-resistant strains, coupled with the organism's ability to form spores that are impervious to standard infection control measures have led to more frequent and severe outbreaks. Despite its rising prevalence, little is known regarding the mucosal immune response to *C. difficile*. We found that *C. difficile*-infected mice develop disease that closely mimics human CDI, including severe diarrhea and weight loss associated with epithelial damage and edema in the cecum and colon. Infected animals succumb within 2 to 4 days or begin to clear the organism and recover shortly thereafter, indicating that innate defenses, and not adaptive immunity, are critical for host protection. We characterized the components of the innate response to CDI and demonstrated, as in human infections, a marked influx of neutrophils into the lamina propria. However, antibody-mediated depletion of neutrophils *in vivo* had only marginal effect on survival. Further analysis of cecum, colon and mesenteric lymph nodes shows rapid and steady up-regulation of IL-17A, coinciding with the infiltration of γδ T cells into the tissues. Intracellular staining further confirms IL-17A is produced predominantly by CD8αα$^-$ RORγτ$^+$ γδ T cell, with only minor contribution from αβ T cells. Since IL-17A is known to have diverse effects in the intestine, including neutrophil recruitment, increased expression of antimicrobial peptides, and epithelial repair, we sought to determine the role of this cytokine in defense against CDI. Infection of *Tcrd-/-* mice (lacking γδ T cells) and *Il17a-/-* mice shows significantly higher mortality than wild-type control, suggesting γδ T cell-derived IL-17A plays a host-protective role during CDI. *Il17a-/-* mice exhibit greater degree of inflammation and tissue pathology following CDI as well as a trend towards greater *C. difficile* burden. Interestingly, no defect in neutrophil recruitment was observed; alternative roles for IL-17A, such as regulation of antimicrobial peptides and genes involved in epithelial repair are currently under investigation. Preliminary analysis of human CDI samples have also revealed the presence of *IL17A* and *TCRD* transcripts, suggesting that IL-17A-expressing γδ T cells may be an important component of the host response in patients with *C. difficile* infection.

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### Cellular Basis of Antibody Titer Maintenance: Heterogeneity of the Bone Marrow Plasma Cell Pool

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Long-lived plasma cells (PCs) are responsible for maintaining antibody titers and are believed to populate unique survival niches in the bone marrow (BM). Current models predict that BM PCs consist chiefly of long-lived, slowly renewing cells. However, we find the turnover rate of the BM PC pool to be much higher than predicted by these models; in fact, more than 50% of BM PCs exhibit characteristics of recently formed PCs. These characteristics include surface expression of the canonical naïve B cell surface protein B220, and a 50% renewal rate of less than 3 days. Surprisingly, despite the rapid turnover rate exhibited by $B220^+$ BM PCs, antigen-induced antibody secreting cells are found within this population for more than 100 days post-immunization. Moreover, upon immunization with a T cell-dependent antigen, the BM PC pools contain significant numbers of antigen-specific low affinity cells, suggesting a T cell-dependent, germinal center (GC)-independent origin. Together these data suggest that BM niches are continuously repopulated by newly generated plasma cells long after antigenic exposure and offer intriguing insights on the identity of the cellular precursors of BM PCs. Funded by NIH AI-097590 and AI-090700.

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### Functional Analysis of Key Genetic Drivers of Cutaneous Squamous Cell Carcinoma

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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 $2 billion. Cutaneous Squamous Cell Carcinoma (cSCC) comprises 15-20% of all skin cancers. cSCC has the best-defined progression from a distinct precancerous lesion, the Actinic Keratosis (AK), to invasive cSCC. Approximately 65% to 72% of cSCC arise in association with AKs. Destructive therapies are the mainstay of AK treatment, but they must be used repetitively with significant morbidity and mortality. Furthermore, in high-risk patients, the sheer multiplicity of lesions makes widespread use impractical. Therefore, there is a tremendous need for rationally designed targeted diagnostics and therapy for AKs, representing an important opportunity for secondary skin cancer prevention. Our knowledge of the molecular and cellular events that lead to sequential transformation of normal skin to AK and subsequently to cSCC is very limited thus representing a fundamental gap in our understanding of events necessary for cSCC progression. Our long-term goal is to identify important genetic events that determine the progression of normal sun-exposed skin (NS) to AK and subsequently to cSCC, and target them for the prevention and therapy of cSCC. We have used a novel approach that combines RNA-Seq, miR-Seq, and reference exome sequencing on tissue-specific, matched samples at three stages of tumor development. The power of our study rests in performing interlesional analysis on internally controlled lesions on the path to carcinoma. Through our initial analysis of 10 sets of matched samples, we have identified miR-181 as a potential molecular target as the expression of the entire miR-181 family gradually increases throughout progression of NS to preneoplastic AK and subsequently to cSCC ($P<0.05$). RNA-Seq profiling identified 13 downregulated transcripts as potential direct targets for the miR-181 family. We hypothesize that upregulation of miR-181 promotes initiation and progression of keratinocyte transformation by targeting TGFBR3. The expression of miR-181 and TGFBR3 as important drivers of AK progression to cSCC will be validated by qPCR and immunohistochemistry. 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 preventing and treating cSCC.

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### Metabolomics of Mammalian Brain Reveals Regional Network Differences

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The mammalian brain is organized into regions with specific biological functions and properties. The metabolome—a collection of small molecule, metabolites—is critical for the proper functioning of the cell. These metabolites are at the intersection of the genetic background of a given cell and the environmental influences that affect it. Thus, they directly reveal information about the physiologic state of a biological system under a particular condition. We hypothesize that different regions of the brain have a set of metabolites that reflects their biological behavior. The objective of this study is to investigate the metabolome of four regions of the mammalian brain: frontal parenchyma, hippocampus, cerebellum, and olfactory bulb. To test our hypothesis, we utilized gas- and liquid- chromatography mass spectrometry platforms and metabolomics approach. From the selected regions, we identified 215 compounds based on a library of 2500 small molecules. Principal component analysis, an unsupervised multivariate analysis, showed four distinct clusters relating to the brain regions, thus providing the unique metabolic profile of each region. We then incorporated a graphical lasso algorithm to determine a binary partial correlation among the identified metabolites. A resulting network of metabolites reveals information that links different metabolic pathways. Through the Kyoto Encyclopedia of Genes and Genomes (KEGG) inquiry, enzymes within these metabolic pathways were identified and further linked to genes associated with neurological pathology. In conclusion, we established a metabolic signature of the selected

regions of mammalian brain, which provide a new perspective on the underlying properties of brain regions. This study has set the foundation for investigating the human brain metabolome.

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## Magnetic Resonance Spectroscopy of Collagen VI Myopathies and Duchenne Muscular Dystrophy

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**Background:** Collagen Type VI myopathies (C6M) and Duchenne Muscular Dystrophy (DMD) are characterized pathophysiologically by muscle inflammation, fatty infiltrate, and fibrosis. Current assessment of disease progression, or conversely, therapeutic improvement, is limited to functional testing or muscle biopsies. With Magnetic Resonance Spectroscopy (MRS), we demonstrate the ability to quantify metabolites, lipids, and water within muscle non-invasively. **Methods:** MR scans of the right lower leg of 17 C6M subjects, 14 DMD subjects, and 11 healthy controls were imaged at the University of Florida on a 3T whole-body MRI scanner either using a SENSE 8-channel or a transmit 16 coil receive knee volume coil. $^{1}$H-MRS was acquired using single voxel STEAM from the soleus muscle, and $T_2$ MRS values were derived using non-linear spaced TE's using PCA, and kinetics were resolved with mono-exponential and non-linear least squares analysis. Spectra were processed using AMARES in jMRUI to calculate total water, intra-myocellular lipid (IMCL), extra-myocellular lipid (EMCL), trimethylamine (TMA), total creatine (TCr), $T_1$ and $T_2$ values of $^{1}H_2O$, and muscle Fat Fractions corrected for partial saturation (FF) and $T_2$ relaxation. **Results:** Mean ages of control, C6M, and DMD subjects were 26±15, 23±17, and 10±2 years, respectively. FF were calculated to be 0.04±0.03, 0.15±0.11, and 0.52±0.22 for control, C6M, and DMD subjects, respectively ($p<0.05$). DMD displayed higher IMCL:TCr and IMCL:Water values versus Control and C6M subjects ($p<0.05$). TCr:water ratios were significantly increased in both C6M and DMD subjects versus controls. Significantly increasing $T_2$ (28.4±0.9 ms, 29.8±1.3 ms, and 31.7±2.3 ms) and $T_1$ (1358±49 ms, 1400±51 ms, and 1461±91 ms) times were measured in control, C6M, and DMD subjects, respectively. **Conclusion:** We demonstrate the feasibility of MRS as a novel non-invasive biomarker that may accelerate translational intervention for both diseases. To our knowledge, this is the first investigation of C6M through MRS to analyze the biochemical composition of pathologic muscle.

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## Mitochondria-targeted Antioxidant Ameliorates Glucose Intolerance, Obesity, and Diastolic Dysfunction in Type II Diabetes

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**Backgrounds:** Diastolic heart failure (DHF) accounts for half the cases of heart failure with similar mortality to systolic heart failure. Although the mechanism is unknown, obesity and diabetes are associated with DHF. Diabetes is associated with mitochondrial oxidative stress, and we have shown that oxidative stress leads to DHF. Therefore, we assessed the effect of mitochondria-targeted antioxidant (Mito-TEMPO) on diabetes-induced diastolic dysfunction. **Methods:** C57BL/6J mice were fed either 60 kcal% fat diet (HFD) or 10 kcal% diet (control) for 8 weeks with or without Mito-TEMPO administration from weeks 3 to 8. Each group underwent tissue-tagged cardiac magnetic resonance imaging (CMR) to assess diastolic function and glucose tolerance testing at week 8, prior to heart tissue harvest. **Results:** HFD mice developed obesity and diabetes as evidenced by impaired glucose tolerance compared with the control (serum glucose = 495 ± 45 mg/dL vs. 236 ± 30 mg/dL at 60 min after glucose challenge, $p<0.05$). CMR tagging detected significantly reduced diastolic strain rate in the HFD mice compared with the control (5.0 ± 0.3 1/s vs. 7.4 ± 0.5 1/s, $p<0.05$), indicating significant diastolic dysfunction in the HFD mice. Systolic function was comparable in both groups (left ventricular ejection fraction = 66.4 ± 1.4% vs. 66.7 ± 1.2%, $p>0.05$). This diabetes-induced diastolic dysfunction was correlated with significant mitochondria reactive oxygen species and oxidative damage, glutathionylation and nitrotyrosination of myosin binding protein C. Mito-TEMPO treatment attenuated obesity (body weight = 27.5 ± 0.9 g vs. 33 ± 0.6 g, $p<0.05$) and significantly improved glucose intolerance (serum glucose = 306 ± 36 mg/dL vs. 495 ± 45 mg/dL at 60 min after glucose challenge, $p<0.05$), compared with untreated HFD mice. CMR tagging showed Mito-TEMPO treatment prevented diastolic dysfunction in the HFD mice as evidenced by comparable diastolic strain rate (6.5 ± 0.7 1/s vs. 7.4 ± 0.5 1/s. $p>0.05$) to the control. **Conclusions:** Mitochondria-targeted antioxidant treatment attenuates obesity, improves glucose intolerance, and prevents diastolic dysfunction, suggesting mitochondrial oxidative stress may mediate these conditions in type II diabetes mellitus.

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## Predictive Value of Dynamic Cancellation Testing to Inpatient Rehabilitation Outcomes

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**Objective**: Pen-and-paper cancellation tests have been shown to predict rehabilitation outcomes, but without evaluating their dynamic performance aspects. In this preliminary exploratory study we evaluated the sensitivity of two different cancellation test methods, including computerized dynamic measures,

to rehabilitation outcomes. We hypothesized that dynamic measures are superior to standard accuracy scores. **Participants and Methods**: 13 adults with acute brain injury (stroke, TBI) and 2 hospitalized patients without brain injury completed the Star Cancellation Test 4 times each on both a touchscreen computer and sheets placed on a graphics tablet. Test method was randomized across subjects. Software automatically calculated the number of contacted targets, non-targets, and blank areas between stimuli and search organization measures, except that marking accuracy on paper tests was judged by 2 raters blinded to patients' identities. The Functional Independence Measure (FIM)—a standard assessment of basic self-care skills—was measured at admission and discharge, and the FIM efficiency (average FIM change per hospitalization day) was calculated. We then correlated cancellation measures to FIM efficiency. **Results**: Neither touchscreen nor paper tests were significantly correlated with FIM efficiencies. However, certain dynamic subtests on the touchscreen method were moderately correlated with FIM efficiency: target marking speed ($r = 0.45$) and search organization ($r = 0.4$). **Conclusions**: We preliminarily suggest that dynamic aspects of touchscreen computer testing may be most sensitive to rehabilitation outcomes. Touchscreen may be superior to paper testing because it is more affected by disturbances of motor control as well as self-organization, both of which are important to functional recovery. Our findings will be reevaluated in larger patient samples. Because prior work has shown that cancellation tests can be completed by the most language-impaired adults, the results may eventually lead to a novel way to evaluate mechanisms important to functional recovery in the most severely language-compromised patients.

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## Alzheimer's Disease Frontal Cortex Exhibits Chromatin Alterations Characteristic of Senescence

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Aging is a major risk factor for Alzheimer's disease (AD); however, the aspects of the aging process that predispose the brain to the development of AD are largely unknown. One potential mechanism is the induction of the senescence phenotype. Human astrocytes undergo senescence in response to stress and this response could be physiologically relevant given that we are able to detect senescent astrocytes in AD and aged brain tissues. The histone variant macro H2A accumulates in senescent cells and in tissues of aged animals, yet little is known about chromatin alterations associated with aging and neurodegeneration in human brain tissue and the functional impact of these changes on the pathogenesis of AD. In order to begin to assess the frequency and significance of chromatin alterations characteristic of senescence during AD, we measured the nuclear staining intensity of macro H2A in postmortem brain tissues and in senescent astrocytes *in vitro* using immunofluorescence. The level of macroH2A was increased in the frontal cortices of AD subjects versus age- and sex-matched controls and in senescent versus pre-senescent human fetal astrocytes. These findings suggest that chromatin alterations characteristic of the senescent phenotype may underlie AD pathogenesis.

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## Potential Toxicity of the Sialic Acid Neu5Gc to the Vertebrate Brain

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The sialic acids *N*-acetylneuraminic acid (Neu5Ac) and *N*-glycolylneuraminic acid (Neu5Gc) are sugars commonly found at the terminal ends of glycosylated structures in vertebrates. In most tissues, the proportion of total sialic acid represented by Neu5Gc is highly variable when compared across species. The sole exception to this is the vertebrate brain, which, despite containing the highest concentration of Neu5Ac of any vertebrate tissue, also contains strikingly low levels of Neu5Gc (<3% in all vertebrate species tested to date). The expression of Neu5Gc's synthetic enzyme, CMP-Neu5Ac hydroxylase (CMAH), is correspondingly very low in the brain. To our knowledge no other molecule demonstrates this distribution, being highly expressed throughout most tissues but exhibiting evolutionarily conserved suppression of expression in the brain alone. This unusual distribution suggests that Neu5Gc presence may exert a detrimental effect in brain. We hypothesized that the presence of Neu5Gc may interfere with the degradation of brain glycoconjugates, and recently proposed a candidate mechanism by which this effect might occur. The important brain glycan polysialic acid (polySia, commonly found as PSA-NCAM) is degraded less effectively by vertebrate neuraminidase in the presence of Neu5Gc. In the current study, we use mouse models of Neu5Gc overexpression to characterize the effects of Neu5Gc overexpression in the brain. Initial efforts using CMV- and NSE-driven transgenes resulted in high embryonic lethality. However, the use of the lox-Cre system to create inducible, tissue-specific expression of *Cmah* has allowed us to circumvent this problem. Surprisingly, lethality was not observed with the inducible transgene, likely due to a difference in the timing of transgene expression during embryonic development. The resulting viable mice express high levels of brain Neu5Gc, with diffuse expression throughout the parenchyma. Here we further characterize the phenotypes and abnormalities in brain glycosylation of these models of *Cmah* overexpression. These models will provide an important foundation for further understanding the unusual universal exclusion of Neu5Gc from the vertebrate brain.

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

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The production of hematopoietic cells in the bone marrow is tightly and dynamically regulated in response to environmental stimuli. In response to stress, neutrophil production is markedly increased

while lymphopoiesis is decreased. The mechanisms mediating the shift from lymphopoiesis to granulopoiesis are unclear, and better understanding the factors regulating this transition may provide novel strategies for sensitizing lymphoid malignancies to chemotherapy. Here we report that exogenously-administered granulocyte colony-stimulating factor (G-CSF), a cytokine induced during infection and a key regulator of granulopoiesis, is associated with marked suppression of B lymphopoiesis in murine bone marrow. After 5 days of G-CSF treatment (250 µg/kg), total B cells in the bone marrow were reduced 8.1 ± 0.9-fold. Analysis of B cell subpopulations (from the most immature Fraction A cells to the most mature Fraction F cells) in the bone marrow showed varying degrees of suppression of all stages of B cell development but normal numbers of the upstream common lymphoid progenitor (CLP). Increased apoptosis of Fraction F B cells in the bone marrow was observed. We also observed mobilization of early fraction B cells to the spleen where they exhibit high rates of apoptosis. Studies of 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 decreased the expression of multiple B-supportive factors in the bone marrow microenvironment including CXCL12, interleukin-6, interleukin-7, and B cell activating factor (BAFF). We also observed 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 mineralizing osteoblasts) resulted in an isolated loss of Fraction F cells in the bone marrow. Deletion of *Cxcl12* using *Osx-Cre* (targeting CAR cells) resulted in loss of bone marrow B cells beginning with Fraction A cells. Deletion of *Cxcl12* using *Prx1-Cre* (targeting mesenchymal progenitors) resulted in severe suppression of B lymphopoiesis that included a loss of CLP. Interestingly, treatment of *Prx1-Cre Cxcl12*$^{flox/-}$ mice with G-CSF resulted 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.

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## Glutamine Addiction: A Novel Targetable Hallmark of HER2/*neu* Breast Cancer Recurrence

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Breast cancer is the most commonly diagnosed malignancy in women and is the second leading cause of cancer-related death in women in the U.S. Among women with breast cancer, tumor recurrence represents the principal cause of mortality. Nevertheless, little is known about the molecular mechanisms underlying how breast cancer cells survive therapy and ultimately recur. In particular, while dysregulated metabolism has long been recognized as a key feature of cancer development, the metabolic changes accompanying cancer recurrence are largely unexplored. To address this gap, our laboratory has developed a series of inducible bitransgenic mouse models that accurately recapitulate human breast cancer progression, including primary tumor development, minimal residual disease, tumor dormancy and recurrence. Increased glutaminolysis has been previously shown to be a key feature of tumorigenesis. To date, no association has been established between glutamine metabolism and breast cancer progression. In this study, we investigated the glutaminolytic differences between primary and recurrent mammary tumors and assess their role as a potential therapeutic target. We found that recurrent tumors exhibit higher glutamine uptake and glutamate production, compared to primary tumors. $^{13}$C-labeling experiments suggested increased glutaminolytic activity and increased reductive carboxylation in tumor recurrence. The observed changes in the glutaminolytic profile were accompanied by a Myc-dependent increased expression of the glutamine transporter, Slc1a5, as well as increased expression of the enzyme that catalyzes the conversion of glutamine to glutamate, glutaminase (Gls1), in recurrent tumors. In vivo orthotopic tumor growth assays revealed that both Slc1a5 and Gls1 expression are required for recurrent, but not primary tumor growth. Human association studies further showed an association between increased *SLC1A5* expression and decreased recurrence-free survival in breast cancer patients, highlighting the potential translational potential of these findings. Combined, our results suggest that recurrent HER2/*neu* mammary tumors are glutamine-addicted. Targeting glutamine metabolism might be a promising therapeutic strategy for the treatment of breast cancer recurrence.

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## Decreased SIRT3 in B Cell Malignancies Confers Growth Advantage

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**Introduction**: Chronic lymphocytic leukemia (CLL), the most common adult leukemia, is characterized by the expansion and accumulation of functionally defective CD5+ B cells in the blood, bone marrow, and lymphoid organs and tissues. Sirtuin 3 (SIRT3) is the major deacetylase within the mitochondrial matrix and regulates the acetylation state of many mitochondrial proteins. SIRT3 has been shown to deacetylate and thereby activate isocitrate dehydrogenase 2 (IDH2) and superoxide dismutase 2 (SOD2). The actions of SIRT3 promote aerobic respiration and prevent oxidative damage. Because cancer cells utilize aerobic glycolysis and have higher reactive oxygen species (ROS), we hypothesized that CLL cells will express lower levels of SIRT3. **Methods**: CLL cells and normal B cells were collected with IRB approval from peripheral blood of CLL patients and healthy donors, respectively. The following B cell malignancy lines were analyzed: JeKo-1, Mino, Raji, Rec-1, RPMI-8226, SUP-B15, U266, Z-138. Mitochondrial lysates were used for biochemical analysis of SIRT3, IDH2, and SOD2. Western blots were used to quantify protein. Spectrophotometric assays were used to measure enzyme activities. Staining cells with dihydroethidium and CFSE and subsequent flow cytometric detection were used to measure ROS and cell proliferation, respectively. **Results**: SIRT3 protein expression was significantly reduced in the 10 CLL patient samples and a number of B cell malignancy lines compared to primary B

cells from healthy donors. Furthermore, loss of SIRT3 correlated with higher levels of acetylated IDH2 and SOD2 and lower IDH2 and SOD2 activities. Less active IDH2 produces less NADPH. With lowered NADPH and less active SOD2, ROS elimination is inhibited. Indeed, we found higher ROS levels in the CLL cells compared to normal B cells. Lastly we demonstrated that SIRT3 overexpression could partially reverse cancer phenotypes in a SIRT3-deficient Raji cell line. Overexpressing SIRT3 in Raji cells led to deacetylation and reactivation of IDH2 and SOD2. SIRT3-expressing cells also displayed decreased ROS and decreased proliferation. **Conclusion**: In this first investigation of the role of SIRT3 in hematological malignancies, we have shown that loss of SIRT3 confers a growth advantage to B cell malignancies consistent with enhanced ROS production and the Warburg effect, whereby cancer cells undergo aerobic glycolysis. This suggests that activating the SIRT3 pathway could be targeted therapeutically in treating B cell malignancies, such as CLL.

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## Evaluation of a Commercial Real Time PCR Assay for Detection of Environmental Contamination With *Clostridium Difficile*

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**Background**: Contaminated environmental surfaces are an important source for transmission of *Clostridium difficile*. However, there are currently no efficient and easy to use methods to assess the effectiveness of environmental disinfection. **Methods**: We tested the hypothesis that a commercial real-time polymerase chain reaction (PCR) for the toxin B gene *tcdB* (Xpert® *C. difficile*, Cepheid) would provide a sensitive and efficient method to detect toxigenic *C. difficile* in the environment. Pre-moistened swabs and gauze pads were used to culture high-touch surfaces (toilet seat/hand rail, table/bed rail, phone/call button) in *C. difficile* infection (CDI) rooms before and after post-discharge cleaning by housekeeping. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of PCR from swabs was compared to toxigenic culture by direct plating of swabs. **Results:** Of 22 CDI rooms, 9 were sampled before and 13 were sampled after post-discharge cleaning. One or more *C. difficile* swab cultures were positive for 6 of 9 active CDI rooms and 6 of 13 (46%) cleaned rooms. PCR testing of swabs was specific but had low sensitivity in comparison to culture. Increasing the PCR cycle threshold (Ct) value to 45 increased sensitivity without decreasing specificity. PCR positivity correlated with greater levels of contamination (5/5 sites positive when ≥10 colonies (range: 11-215) present versus 0/6 when < 10 colonies recovered ($P$<0.001). In comparison to swabs, gauze cultures had 50% higher site positivity in both active CDI and cleaned rooms. **Conclusions**: In comparison to culture, we found that a commercial PCR assay had good sensitivity for detection of heavy environmental contamination, but poor sensitivity for detection of low levels of contamination that were typically present after rooms were cleaned. Modifications of the assay such as lowering the PCR Ct or increasing the surface area sampled may result in improved sensitivity.

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## Identification of ERβ-regulated Genes in Endometriosis

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Endometriosis is an estrogen-dependent gynecological disease that affects 6-10% of women of reproductive age. It is a major cause of chronic pelvic pain and infertility, and poses a heavy financial burden on society, with annual estimates totaling $22 billion in the US alone. Methylation defects have been characterized in stromal cells derived from ovarian endometriotic lesions. In particular, a CpG island in the ESR2 promoter region is hypomethylated and contributes to a corresponding increase in ERβ mRNA and protein relative to the normal endometrium. The high local levels of 17 β-Estradiol ($E_2$) that are present in the endometriotic lesion milieu underscore the importance of elevated ERβ expression in endometriosis. However, the precise contribution of ERβ to endometriosis has not been fully characterized. In this study we show that ERβ transcriptionally regulates a subset of genes whose corresponding proteins possess intrinsic kinase or GTPase activity and contribute to endometriotic cell proliferation and survival. We correlated the differentially expressed genes from a microarray that compared normal endometrial and endometriotic stromal cells with previously identified ERβ genomic targets from ChIP-on-ChIP and ChIP-Seq experiments. Using this strategy, we found 70 differentially expressed genes in endometriosis that are potentially regulated by ERβ, 42 of which encode phosphoproteins. We validated that two of these genes are in fact ERβ genomic targets by showing 1) that their transcript and protein levels increase in response to $E_2$, 2) that their expression decreases after ERβ siRNA knockdown, and 3) that ERβ is enriched at the promoter regions of these two genes. Furthermore, we showed that these two genes, ras-like and estrogen regulated growth inhibitor (RERG) and serum and glucocorticoid-regulated kinase-1 (SGK-1), contribute to cell proliferation and apoptosis in endometriotic stromal cells. Our results demonstrate that in endometriosis, ERβ regulates a novel and important network of genes with intrinsic kinase and GTPase activity that control cell fate. Moreover, our study underscores the contribution of an altered nuclear receptor to endometriosis and poses ERβ as a potential drug target for this debilitating disease.

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## Investigating the Two-pronged Role of HEYL in Breast Cancer Angiogenesis

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**Background/Aim/Hypothesis**: HEYL is a transcription factor that is over-expressed in breast cancer cells and breast cancer associated endothelial. HEYL regulates the expression of genes involved in angiogenesis and invasion, like secreted CXCL1, 2 and 3. The aim of our study was to define the angiogenic role of HEYL in breast cancer cells and breast cancer associated endothelial cells, and to find a marker of HEYL activity amenable to *in vivo* imaging. **Methods**: Using conditioned media from HS578T breast cancer

cell lines +/- HEYL expression, migration assays and tube-forming assays were performed with human umbilical vein (HUVEC) and human mammary microvascular endothelial cells (HMMEC) to determine whether HEYL could stimulate angiogenesis through the upregulation of paracrine signaling molecules like CXCL1, 2 and 3. HEYL/HER2 double transgenic mice and HER2 transgenic mice were allowed to form spontaneous tumors, and these tumors were stained with CD31 to quantify vessel number, length and area. HMMECs +/- HEYL expression were used in invasion assays and ECM remodeling assays to determine HEYL's effect on the invasion and remodeling abilities of endothelial cells. HEYL knockout mice were created to test the migration ability of neonatal mouse retinal vessels lacking HEYL. Claudin 1 western blot assays were performed in breast cancer cell lines with HEYL siRNA knockdown or HEYL deletion mutants. **Results**: Media conditioned by a HEYL overexpressing breast cancer cell line caused increased tube forming and migration of endothelial cells. Migration was decreased when anti-CXCL1/2/3 antibodies or CXCR2 inhibitor were added to the media. HEYL/HER2 tumors had a greater vessel density and grew more rapidly than HER2 tumors. HEYL overexpression in HMMECs led to greater invasion and ECM remodeling. In HEYL knockout mice, retinal vessels had decreased migration distance compared to wild type mice. Claudin 1 expression decreased with HEYL siRNA knockdown and with HEYL deletion mutants. **Conclusions**: HEYL expression increases the secretion of CXCL1/2/3 by breast cancer cells, which act on the endothelial CXCR2 receptor to increase angiogenic potential. HEYL expression in endothelial cells can increase angiogenic potential *in vitro* and *in vivo*. Claudin 1 expression is a marker of HEYL activity.

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## CEP290 Directly Links Microtubules to Membrane, and it's N- and C-Termini Inhibit Protein Function

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**Purpose:** Little is known about the functional domains of the protein CEP290, a protein critical to the function of the primary cilium, encoded by the gene most commonly associated with the devastating blinding disease Leber Congenital Amaurosis (LCA). This study aimed to identify different functional regions of the protein to better understand and define the regions necessary for protein function. **Results:** The N-terminus of CEP290 was found to be necessary and sufficient for direct membrane-binding, and a 300 amino acid region near the C-terminus of the protein was found to be necessary and sufficient for microtubule binding and stabilization. Microtubule binding was found to be deficient in the rd16 mouse disease model, which produces a truncated version of Cep290 lacking much of the microtubule binding region. Using microtubule and membrane binding as functional readouts of CEP290 activity, regions of the protein were identified which cooperate to inhibit protein function. Overexpression of these inhibitory regions in cells with endogenous CEP290 resulted in the dysregulation of the protein, leading to the aberrant formation of primary cilia. **Conclusions:** The ability of CEP290 to bridge the gap between the microtubule axoneme and the ciliary membrane has long been postulated to be a critical role in the protein's function. Our data show this hypothesis to be correct, and sharply define the regions of the protein responsible for these activities. Furthermore, the identification and validation of novel inhibitory domains of CEP290 present the possibility that a truncated protein lacking these inhibitory regions might retain the functionality of the full length gene. Such a mini-gene could be used therapeutically for the treatment of LCA due to *CEP290* mutations, and may be small enough to fit the limited cargo capacity of AAV gene therapy vectors.

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## Interrogating DNA Methylation Differences in Endometriosis Identifies a GATA Switch

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Endometriosis causes chronic pain and infertility in 10% of reproductive-age women due to the extrauterine growth of endometrial-like cells. These cells respond to estrogen but not progesterone signaling, and are characterized by increased inflammation and resistance to apoptosis. DNA methylation is speculated to affect the etiology of endometriosis, but only a handful of genes implicated in the pathology of endometriosis are differentially methylated. Using Illumina's high resolution 450K methylation array in concert with their HT12v4 gene expression array, we uncovered significant, focused differences in methylation between healthy human endometrial and endometriotic stromal cells that correlated with both baseline and steroid hormone-induced differences in gene expression. Interaction analysis based upon the context of differentially methylated loci to adjacent CpG islands and transcriptional start sites identified a subset of genes enriched in transcription factors that regulate urogenital development, female sexual development, and steroid hormone response. In addition to confirming and expanding the methylation profiles of genes known to be differentially methylated endometriosis, such as NR5A1 and HOXA11, this provided a novel set of epigenetically regulated genes including several GATA family members that misdirect the differentiation of endometriotic cells. Functional analysis of the GATA family in primary cells revealed GATA2 is necessary for the hormone-driven differentiation of healthy stromal cells, but this gene is hypermethylated and repressed in endometriotic cells. In contrast GATA6, which is hypomethylated and abundant in diseased cells, potently blocks hormone sensitivity, represses GATA2, and induces markers of endometriosis when expressed in healthy cells. This data uncovers a unique epigenetic fingerprint in endometriosis, and reveals how focused changes in DNA methylation underlie the phenotypic defects in the disease. Moreover, this work identifies a novel role for the GATA family as key regulators of uterine physiology, suggesting that aberrant methylation in endometriotic cells underlies a shift in GATA isoform expression that facilitates progesterone resistance and disease progression.

# Poster Abstracts

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## Genes Required for Survival by *Salmonella* Typhimurium During Intestinal Inflammation

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Non-typhoidal *Salmonellae* is the leading cause of bacterial foodborne disease in the U.S.A, and *Salmonella* enterica serotype Typhimurium is the most frequently isolated serotype. The immune response to the organism is a large neutrophilic infiltrate and an increase in mucus and anti-microbial peptide production from intestinal epithelial cells. To induce the large inflammatory response *S.* Typhimurium utilizes a Type Three Secretion System (TTSS-1) to inject effectors into host epithelial cells. Use of this system also induces uptake of *Salmonellae* by intestinal epithelial cells. The inflammatory response ultimately alters the host microbiota while *S.* Typhimurium is relatively resistant to it. Using pools of mutants bearing targeted gene deletions of either individual genes (single-gene deletion or SGD) or multiple contiguous genes (multi-gene deletion or MGD) of the STm genome we have identified mutants under selection in bovine ligated ileal loops. In addition to genes previously known to be important for STm survival during inflammation in the intestine, we have identified many novel genes previously unknown as critical for survival in the intestine. Two mutants, ΔSTM1188 and ΔSTM4509.s, were confirmed in ligated ileal loops as less fit during competitive infections with isogenic wild type. In conditions where a neutrophilic inflammatory response is absent, double mutants missing either STM1188 or STM4509.s as well as SPI-1 were not under selection in competitive infection with isogenic wild type. Deletion mutants in STM1188 and STM4509.s were further evaluated using the streptomycin-treated mouse colitis model and both had reduced fitness. Fitness was rescued when the genes were present in trans during competitive infections with the wild type organism in this model. Interestingly, the human host-adapted serovar *S.* Typhi, which possesses a degenerate chromosome and does not induce an intestinal inflammatory response, lacks the STM1188 gene. Because *S.* Typhi does not trigger a large innate immune response this gene was presumably not required for survival and subsequently lost from the genome. Early investigations into the molecular role of STM1188 indicate the promoter is induced during the presence of the cationic anti-microbial peptide, polymyxin B, and during low oxygen conditions, further indicating a role for STM1188 in the presence of inflammatory anti-microbial peptides.

## 65

## PD-1 Contributes to Respiratory Virus Reinfection by Impairing Memory CD8$^+$ T Cells

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Viruses are leading causes of severe acute lower respiratory infections (LRIs). These infections evoke incomplete immunity, as individuals can be repeatedly reinfected throughout life despite the presence of neutralizing antibodies. We previously found that acute viral LRI caused rapid pulmonary CD8$^+$ cytotoxic T lymphocyte ($T_{CD8}$) functional impairment via programmed death–1/programmed death ligand–1 (PD-1/PD-L1) signaling, a pathway previously associated with prolonged antigenic stimulation during chronic infections and cancer. PD-1-mediated impairment occurred as early as day 7 in the respiratory tract. We employed two different approaches to study the secondary memory $T_{CD8}$ response to human metapneumovirus (HMPV) infection: a) infection of μMT mice (which lack B cells) and subsequent reinfection, and b) immunization with single HMPV $T_{CD8}$ epitopes to mimic potential vaccination strategies and challenge infection. We demonstrate that memory $T_{CD8}$ become impaired more rapidly and to a greater degree than naïve $T_{CD8}$ responding to primary infection. PD-1 was rapidly re-expressed on all secondary effector T cells. Therapeutic blockade of PD-1 signaling with anti-PD-L1 monoclonal antibody potently restored function to the impaired pulmonary $T_{CD8}$, resulting in enhanced viral control. Furthermore, we generated WT:PD-1$^{-/-}$ bone marrow chimeras to show that PD-1 plays a $T_{CD8}$-intrinsic role in mediating impairment in the respiratory tract. These results suggest that PD-1 causes $T_{CD8}$ functional impairment during reinfection or challenge infection and may contribute to recurrent viral LRIs. Therefore, the PD-1/PD-L1 pathway may represent a therapeutic target in the treatment and prevention of respiratory virus infections.

## 66

## Imbalance in NGF/proNGF Ratio as Biomarker of Diabetic Retinopathy

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**Background:** Preclinical studies have demonstrated that diabetes-induced oxidative stress can alter the homeostasis of retinal nerve growth factor (NGF) resulting in accumulation of its precursor, proNGF at the expense of NGF. This imbalance was aggravated with duration of diabetes and coincided with retinal damage in experimental diabetes. Here we test the hypothesis that alteration of NGF and proNGF levels observed in the retina will be mirrored in the serum of diabetic mice. **Methods:** Western Blot analysis was performed on retinal and plasma samples collected from C57Bl/6 mice that were kept diabetic for 5- weeks using STZ-model. Results: proNGF expression was increased in diabetic mouse plasma and retina. Diabetes increased proNGF levels to 2.25 fold of the control levels in both retina and plasma of the same animals. NGF expression was attenuated in diabetic mice to 50% and 60% in retina and plasma of the same animals, respectively. **Conclusion:** Our results showed that the diabetes-upregulated expression of proNGF and impaired NGF expression was comparable between retina and serum. Pharmacological regulation of NGF/proNGF homeostasis may have therapeutic potential in the clinical management of diabetic retinopathy. Translational Impact: NGF plays an important role in improved wound healing and inflammatory responses, and preserving retina function. Further characterization of the imbalance of proNGF to NGF ratio may facilitate its utility as an earlier and more accurate

biomarker for diabetic complication and in particular diabetic retinopathy.

## 67

## Tenofovir, a Potent Anti-viral Agent, is an Ecto-5'Nucleotidase (CD73) Inhibitor Which Decreases Adenosine Production to Prevent Dermal Fibrosis in a Murine Model of Scleroderma

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**Background:** Acyclic nucleoside phosphonates are a key class of antivirals commonly used in the treatment of both DNA and retroviral infections. Adefovir and tenofovir are AMP analogues that resemble substrates of CD73. We have previously reported that adenosine, generated by the CD73-mediated dephosphorylation of AMP, plays a critical role in development of both hepatic and dermal fibrosis in murine models of cirrhosis and scleroderma, respectively. A recent clinical trial demonstrated that tenofovir, but not other antiviral agents, reverses hepatic fibrosis/cirrhosis in patients with hepatitis B. We therefore proposed that tenofovir's antifibrotic effects are mediated by inhibition of adenosine production by CD73-mediated dephosphorylation of AMP. **Methods:** In silico modeling was performed using an ICM-Browser downloaded from www.molsoft.com. CD73 enzyme activity was quantitated by malachite green using 75-100 uM AMP substrate. Bleomycin (0.25 U, SubQ)-treated mice were treated with vehicle, Adefovir, or Tenofovir (75mg/kg, IP) [n=5 per group]. Skin breaking strength was measured using a tensiometer. With SigmaScan software, scar index was determined as the ratio of red/green pixels representing compact/filamentous fibers. Hydroxyproline content was quantified by colorimetric assay. Adenosine levels were analyzed with HPLC. **Results:** In silico modeling data suggested that both adefovir and tenofovir bound to the enzymatic pocket of CD73. Tenofovir, but not adefovir, inhibited CD73 activity of 293T cells overexpressing CD73 (38$\pm$7.4%, at 10 uM) and of recombinant enzyme (72$\pm$1.0%, at 10uM). Tenofovir significantly decreased adenosine levels in the skin of bleomycin-challenged mice (273.95$\pm$8.41 vs. 432.58$\pm$24.34nM adenosine/12mm punch biopsy, n=8-10, [$p<0.05$]). Tenofovir (75mg/kg) diminished bleomycin-induced dermal fibrosis in bleomycin-treated mice (73.7$\pm$3.1% reduction of hydroxyproline content [$p<0.05$]; 33.5$\pm$3.8% reduction of dermal thickness [$p<0.05$] and reduction of breaking tension by 66.8$\pm$1.4% [$p<0.05$]). Picrosirius red staining showed diminished dense collagen fibrils (scar index of 1.2$\pm$0.1 vs 22.2$\pm$0.7 [$p<0.001$], normal skin is 2.5). **Conclusions:** These results provide strong support to the hypothesis that Tenofovir reduces fibrosis via inhibition of adenosine production from AMP by CD73. Moreover, these results suggest that tenofovir may have therapeutic potential in treating fibrosis in patients suffering from non-viral fibrosing diseases such as scleroderma.

## 68

## Regulation and Targeting of Fyn in Cutaneous Squamous Cell Carcinoma

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Over 700,000 cases of cutaneous squamous cell carcinoma (cSCC) occur in the United States every year. Despite this high rate of diagnosis, no therapies currently exist that target the molecular mechanism of tumorigenesis. Approximately 50% of these tumors contain mutated Ras; however targeting Ras with therapeutics has been unsuccessful in clinical trials. Expression of active H-Ras (G12V) in HaCaT cells (immortalized, non-transformed human keratinocytes) resulted in dramatic (>100 fold) upregulation of the Src family kinase Fyn. Fyn is overexpressed in many malignancies and is involved in the elevated invasion/migration of HaCaT cells constitutively expressing active Ras (HaCaT-Ras cells). To explore the transcriptional regulation of Fyn, we subcloned varying segments of the human Fyn promoter that span from -2 kb to -50 bp upstream to the transcription start site in front of a firefly luciferase gene and transfected the reporter constructs into HaCaT and HaCaT-Ras cells. We identified the region -100 to -50 bases upstream to the Fyn transcription start site as being required for high Fyn expression. Furthermore, mutation of 2 bases within this segment abolished Fyn over-expression. Electrophoretic mobility shift assays indicate that nuclear proteins from HaCaT-Ras, but not HaCaT cells, bind to this site. Preliminary supershift assays show loss of binding upon the inclusion of a Lef-1 antibody, suggesting this transcription factor may be necessary for Fyn upregulation. We have also targeted Fyn in HaCaT-Ras cells using the Src family kinase inhibitor Dasatinib. Already used to treat some forms of leukemia, Dasatinib is also in clinical trials to treat solid tumors. Treatment with varying concentrations of Dasatinib did not reveal significant differences in cell viability, cell cycle progression, apoptosis or autophagy in transformed HaCaT-Ras cells as compared to HaCaT cells. However, upon Dasatinib exposure HaCaT-Ras cells dramatically changed their morphology, restoring cell-cell adhesion and reverting from a stellate appearance to the more rounded morphology of non-transformed HaCaT cells. Dasatinib treatment also significantly inhibited the ability of HaCaT-Ras cells to migrate in culture. Immunofluorescence staining showed enhanced membrane localization of desmoplakin upon 24 hours of Dasatinib treatment. Therefore, we propose that targeting Fyn in tumors with increased Ras activity will restore cell to cell contacts, reduce cellular migration/metastasis and provide a potential therapeutic target in the treatment of cSCCs.

## 69

## Increased Prevalence of Tracheomalacia in Children with Cystic Fibrosis

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Tracheomalacia is a recognized comorbidity affecting adult patients with cystic fibrosis (CF). However, it is not known whether tracheomalacia represents a congenital condition or is acquired through successive rounds of infection and inflammation. A recent study of the pig model of CF reveals abnormalities of tracheal

morphology at birth that could predispose to tracheomalacia. Therefore, we sought to determine whether tracheomalacia is prevalent among infants and young children with CF prior to the onset of significant infection and subsequent airway remodeling. Using local bronchoscopy records, retrospective analysis revealed that 11% of children with CF in our center also have documented tracheomalacia (n= 12/106). Of these patients, all were pancreatic insufficient and half had meconium ileus. Children with CF and tracheomalacia were noted to have chronic cough and wheezing very early in life, and these symptoms were often poorly responsive to therapy. We also found a surprisingly high incidence of life threatening episodes of airway obstruction among children affected by CF and tracheomalacia. The mechanistic links between loss of CFTR function and airway malacia are currently unknown. We conclude that congenital tracheomalacia is a common and previously unrecognized comorbidity in children with CF that may cause significant pulmonary symptoms in early life.

## 70

## The Role of Galectin-3 Following Alpha-Synuclein Cell Invasion in Parkinson's Disease

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Key to the development of new treatments aimed at arresting Parkinson's disease (PD) progression will be understanding the mechanisms by which misfolded alpha-synuclein (a-syn) can induce dysfunction, how this pathology spreads, and how the neurons respond with innate defense mechanisms. Based on its ability to form an amphipathic alpha-helix and induce membrane curvature, our lab has demonstrated that a-syn gains access to the cytosol by disrupting the endosomal/lysosomal (E/L) membrane. This rupture leads to detrimental consequences such as the generation of reactive oxygen species and the activation of the inflammasome. Galectins are a family of sugar-binding proteins that play a role in diverse cellular processes such as cell signaling and inflammation. Some galectins only recognize sugar moieties normally present on the cell exterior, or interior of E/Ls following endocytosis. It has been demonstrated that these galectins are recruited to ruptured vesicles following E/L lysis by pathogenic bacteria, and play a role in diverse cellular stress responses. Stable overexpression of Gal3 results in the cell-to-cell transfer of Gal3/a-syn complexes. It is known that Gal3 secretion can be induced by activation of caspase-1, an inflammasome effector protein. This transfer could be relevant *in vivo*, and so we hypothesized that Gal3 is involved with a secretory pathway that acts to rid the cell of a-syn that overwhelms the autophagic machinery. In order to test this, we co-cultured N27 a-syn cells with N27 Gal3 cells and measured levels of Gal3 in cell lysate and supernatant samples by western blot following varying a-syn insults. Our results showed Gal3 release from treated cells, but yielded a size difference between supernatant and cell lysate Gal3 that raised the suspicion that cleavage of Gal3 may play a role in the Gal3/a-syn secretion pathway. Phosphorylation of a-syn is an important consequence of mitochondrial dysfunction that influences aggregation, and has been correlated with worsening PD pathology. To determine if Gal3 plays a role in influencing a-syn phosphorylation, wild-type SY5Y and SY5Y Gal3 cells were subjected to varying insults and examined by immunocytochemistry. Our results indicated that with the combined insult of LPS and a-syn aggregates, there were substantially more ~p-a-syn puncta in the Gal3 overexpressing cell compared with the normal control. These results indicate that Gal3 expression is correlated with higher levels of a-syn phosphorylation, and suggest a role for Gal3 in the pathogenesis of PD self-propagation.

## 71

## Alpha-synuclein Induces the Rupture of Endosomes/Lysosomes, Leading to the Generation of Reactive Oxygen Species in Cells

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Recent studies have demonstrated that alpha-synuclein forms an amphipathic alpha-helix with the ability to curve and rupture experimental liposomes. This ability is very similar to protein VI (pVI) from adenovirus. During adenovirus cell entry, pVI disrupts endosome/lysosome (E/L) membranes to allow for the release of the virus into the cytoplasm. This membrane rupture leads to activated lysosomal cathepsins into the cytoplasm, leading to an increase in mitochondrial reactive oxygen species (ROS) and inflammasome activation in infected cells. We observe that alpha-synuclein can induce the recruitment of Galectin 3 (Gal3) to E/Ls, where alpha-synuclein appears to induce curvature of these membranes. Live cell imaging of Gal3/alpha-synuclein complexes demonstrate that these complexes are highly dynamic and can be transferred between cells. As E/L rupture by adenovirus has been shown to induce ROS generation in target cells, we also determined if alpha-synuclein can induce ROS in target cells. Indeed, we observe an increase in ROS in cells treated with alpha-synuclein aggregates or expressing high levels of alpha-synuclein. This alpha-synuclein dependent ROS induction is prevented with cathepsin inhibitors, suggesting that ROS induction by alpha-synuclein required activated cathepsin molecules that leave the vesicular compartment following E/L rupture. We propose that alpha-synuclein induced E/L rupture creates a positive feedback loop that facilitates the evolution of pathological symptoms associated with PD.

## 72

## Picrotoxin Dramatically Speeds the Mammalian Circadian Clock Independent of Cys-loop Receptors

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Picrotoxin is widely and specifically used as an open-channel blocker of $GABA_A$ receptors and other members of the Cys-loop receptor superfamily. We find that picrotoxin acts independently of known Cys- loop receptors to dramatically shorten the period of the circadian clock by specifically advancing the accumulation of PERIOD2 protein. We show that this mechanism is surprisingly tetrodotoxin-insensitive, dose-dependent, reversible, GPCR-

independent, effective at speeding single cell oscillations in gene expression and firing rate, and the effect is larger than any known chemical or genetic manipulation. Notably, our results further indicate that picrotoxin's circadian target is common to a variety of human and rodent cell types and tissue but not *Drosophila*, thereby ruling out all conserved Cys-loop receptors and known regulators of PERIOD protein stability. Given that the circadian clock modulates significant aspects of cell physiology including synaptic plasticity, these results have immediate and broad experimental implications. Furthermore, our data point to the existence of an important, unidentified target within the mammalian circadian timing system.

## 73

## Effects of β-hydroxybutyrate and Low-Glucose on Mitochondrial Membrane Potential and Gene expression in Brain Micovascular Endothelial Cells

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The use of a low-carb, high-fat "ketogenic" diet has long been known to alleviate refractory epilepsy in children. Recently, the ketogenic diet has shown efficacy in treating animal models for a variety of neurodegenerative disease along with being protective following a traumatic brain injury or stroke. We evaluated the effects of β-hydroxybutyrate (BHB) and/or low-glucose on brain microvascular endothelial cells (MEC) cultures. Mitochondrial membrane potential (MMP) was measured using the membrane-permanent JC-1 dye. Preliminary data showed a significant increase in MMP in the Standard EC150 Media + 5mM BHB treatment and a significant decrease in the Low-glucose Media + 5mM BHB treatments when compared to Standard EC150 Media. Gene expression studies of mitochondrial energy metabolism and oxidative stress resistance factor related genes were also performed. However, data is not available at time of abstract submission deadline. Although interpretation is limited, it may suggest that a differential availability of energy substrates has a measurable effect on mitochondrial function.

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## Specificity Protein (Sp) 1 Transcription Factor Modulates Long Noncoding RNA Expression in Liver Cancer Cells

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Hepatocellular carcinoma is one of the most prevalent forms of cancer worldwide and it exhibits highly invasive and metastatic properties. Recent studies have shown that of the small proportion of the genome that is transcribed only about 1.4% encodes for protein-coding genes. Of the remaining vast majority of transcripts that do not encode protein, long noncoding RNAs (lncRNAs) have recently gained attention because of their pivotal role in disease. LncRNAs are a class of transcripts longer than 200 nucleotides and have been characterized as having both tumor suppression and oncogenic functions in many types of cancers. Although their mechanisms of action remain largely unknown, many lncRNAs are regulated by transcription factors in a tissue-specific manner. Specificity protein (Sp) transcription factors Sp1, Sp3, and Sp4 are overexpressed in many tumors, and regulate expression of genes required for cancer cell and tumor growth, survival, angiogenesis, and inflammation. Sp proteins are the targets of many conventional and alternative chemotherapeutic drugs, and therefore further study of their functions is of great interest. In this study, we examined the role of Sp transcription factors in regulating lncRNAs in liver cancer cells. Using HepG2 and Huh-7 cells as models, we investigated the effects of Sp downregulation on the expression of several lncRNAs as well as cell growth and survival. Downregulation of Sp transcription factors by RNA interference or by drugs that target these proteins identified a set of lncRNAs in liver cancer cells that are modulated by Sp transcription factors. Further studies are underway to examine the specific functions of these lncRNAs in liver cancer growth and metastasis as well as their utility as diagnostic biomarkers.

## 75

## The Homeobox Transcription Factor VentX is a Critical Regulator of Human Dendritic Cell Differentiation and Maturation

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Dendritic cells (DCs) are professional antigen-presenting cells that play essential regulatory role in the initiation and maintenance of immune response. Broadly implicated in autoimmune diseases and immuno-therapy, the molecular mechanisms underlying development and function of DCs remain to be fully defined. VentX is a human homologue of the Xenopus homeobox gene Vent/Xom of the BMP4 signaling pathway, and was recently defined as a novel hematopoietic transcriptional factor controlling proliferation and differentiation of immune cells. Expression profiling showed that VentX is expressed in a regulated manner during the development of primary DCs. Using an *in vitro* monocyte-derived DC system, we found that ablation of VentX expression in monocytes significantly impaired their differentiation into DCs induced by GM-CSF and IL4 treatment, and that VentX is required for the full maturation of DCs following LPS stimulation. Overexpression of VentX in monocytic cells THP1 accelerated their differentiation towards DCs, and promoted dendritic morphogenesis. Using gain of function and loss of function approaches, we found that VentX controls dendritic cell functions, such as T-cell stimulation. Mechanistically, we showed that knockdown of VentX led to an enhanced autocrine of IL6, and that neutralizing IL6 activity with specific antibody partially rescued differentiation and maturation defects of DCs upon depletion of VentX. The results of our current studies suggested that VentX is a novel regulator of the development and function of DCs, therefore, provided a new foundation for mechanistic and therapeutic exploration of autoimmune diseases and immuno-therapy.

# Poster Abstracts

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### A Barrier to Diffusion of Opsins But Not Peripheral Membrane Proteins at the Connecting Cilium and Disk Rims of Cone Photoreceptor Sensory Cilia

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**Purpose:** to reveal the mechanisms allowing signaling proteins to be concentrated within the outer segment (OS) of cone photoreceptors. Localization and retention of transduction cascade proteins within ciliary signaling compartments are thought to be mediated, in part, by diffusion barriers at the transition zone. In the retina, mutations in cone opsins and other phototransduction cascade proteins often result in their mislocalization from the ciliary cone OS, and can lead to a spectrum of diseases, ranging from color blindness to progressive photoreceptor dystrophy, and eventually blindness. While the mechanisms for protein transport to and retention within the rod OS have received considerable attention, little is known about these processes in cones. Unlike rods, the OS discs of cones are contiguous with the plasma membrane, and therefore retention of opsin would require a diffusion barrier either at the cilium base, as has been demonstrated for primary cilia, or at the disc rim. To address this problem, we examine the diffusion of the transmembrane red cone opsin-EGFP fusion protein and the peripheral membrane protein double geranylgeranyl-EGFP expressed in cones of Xenopus laevis using multiphoton FRAP. **Methods:** transgenic X. laevis tadpoles were created using the REMI method to expressed either red opsin-EGFP or double geranylgeranyl-EGFP exclusively in their cones. Confocal and multiphoton imaging of living cones from isolated retinas cut in chips were used to study the localization and dynamics upon photobleaching of these proteins. Custom built MATLAB routines were used for quantification of the collected images. **Results:** first, we show that although both proteins diffuse laterally in the disc membranes with similar rates, diffusion of opsin-GFP between discs is significantly retarded compared with double geranylgeranyl-GFP and therefore axial equilibration for opsin is delayed. Second, we show that while double geranylgeranyl-EGFP has access to all cone membranes, opsin is excluded from all cone segments except for the OS where it is highly concentrated. **Conclusions:** our results are consistent with cone opsin retention in the OS being mediated by at least two mechanisms. First, there is a selective barrier to free diffusion for trans-membrane proteins located at the end loops between adjacent discs which do not impede the free movement of peripheral membrane proteins. Second, the fact that eventually both proteins equilibrate within the OS but only opsin is completely excluded from the inner segment, hints at the existence of a selective diffusion barrier at the level of the connecting cilium that prevents opsin from leaking out of the OS.

## 77

### Uncovering the Genetic Regulation of an Axon Self-destruct Pathway

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**Background:** Axon degeneration is a specialized self-destruct program that mediates axon breakdown and clearance in development, injury, and disease. Understanding the genetic regulation of axon degeneration will broaden our understanding of nervous system development and may offer paths toward disease intervention. **Methods:** We have developed a lentiviral RNAi-based screening platform to identify genes required for injury-induced axon degeneration in neurons. Primary sensory neurons are treated with shRNA-bearing lentiviral particles and injured by transection. Axon degeneration is quantified by automated microscopy and image analysis. Target genes identified from the primary screen are validated by qRT-PCR and genetic rescue. **Results:** Our screen of ~16,000 mouse genes has yielded a variety of promising candidate genes that are currently being evaluated. The identification and validation of a bona fide axon degeneration signaling protein Sarm1 demonstrates the effectiveness of this approach. Finally, the serendipitous identification sequence "motifs" that predict off-target axon protection may provide unexpected insights into the nature of shRNA off-target effects in neurons.

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### Aurora A kinase is Required for Hematopoiesis

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In acute megakaryocytic leukemia (AMKL), there is a failure of megakaryocytes (MKs) to differentiate, become polyploid and stop dividing. We used an integrated screening approach to identify small molecules and their targets that control the polyploidization and differentiation of normal and malignant megakaryocytes. We identified several small molecule inducers of polyploidy and used siRNA and proteomic target ID approaches to determine the cellular targets of the lead small molecule dimethylfasudil (diMF). Aurora Kinase A (AURKA) was identified as one of the top targets of diMF. AURKA inhibition by diMF or the selective AURKA inhibitor MLN8237 increased MK polyploidy, induced features of differentiation, blocked proliferation of AMKL blasts, and improved survival in an AMKL mouse model. AURKA is required for embryonic development, however the extent to which AURKA is necessary for steady state hematopoiesis in adults is unknown. To investigate the necessity of AURKA in hematopoiesis, we utilized a conditionally targeted strain of mice (*Aurka*$^{flox/flox}$). We first deleted AURKA in megakaryocytes *ex vivo*, and found that deletion of AURKA resulted in increased CD41 and CD42 expression as well as increased DNA content. Assays for apoptosis by Annexin V staining also showed increased apoptosis in AURKA-deleted cells at 24 and 48 hours. We next deleted AURKA *in vivo* and found that deletion of AURKA in hematopoietic progenitors leads to pancytopenia, profound bone marrow defects and death within two weeks. Colony formation

assays showed significantly decreased myeloid, erythroid and megakaryocyte colony formation with AURKA deficiency. Bone marrow histology displayed markedly hypocellular marrow, but curiously, flow cytometry revealed a significant increase in the percentage of CD41 and CD42 positive cells. Together, our data support a role of AURKA in megakaryocyte polyploidization and differentiation and show that AURKA is required for steady state hematopoiesis. The results also show that AURKA is the key target of diMF in the induction of polyploidization of megakaryocytes and support the development of Aurora A kinase inhibitors in clinical trials for AMKL.

## 79

## BK Channel Modulation in the Treatment of Experimental Asthma

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**Introduction:** Large conductance, voltage and calcium activated potassium (BK) channel are highly expressed in airway smooth muscle (ASM). Here, we describe rottlerin, a potent BK channel agonist, as a potential therapeutic for asthma possessing both anti-inflammatory and smooth muscle relaxing properties. **Methods:** Invasive airway measurements were obtained in ovalbumin (OVA) and house dust mite (HDM) sensitized mice administered rottlerin 5 µg/g body weight every other day via IP injections during the challenge period or as a single IV injection. Small airway contractility and intracellular calcium oscillations were assessed using *ex vivo* lung slice techniques. Whole-cell ruptured patch-clamp technique was used to determine the effects of rottlerin on BK channel currents in airway smooth muscle cells. **Results:** Systemic administration of rottlerin during the challenge period reduced methacholine-induced airway hyperreactivity (AHR) in both the OVA- and HDM-sensitized mice (47% decrease in peak airway resistance in rottlerin-treated OVA-asthma animals as compared to PBS-treated OVA-asthma animals, n=20 per group, $P<0.01$; 54% decrease in peak airway resistance in rottlerin-treated HDM-asthma animals, n=9-10 per group, $P<0.05$). Rottlerin reduced inflammatory cells and Th2 cytokines in bronchoalveolar lavage fluid. A single dose of rottlerin acutely inhibited AHR in the OVA asthma mice (45% reduction in peak airway resistance, n=10 per group, $P<0.05$), suggesting a direct effect of rottlerin on ASM contractility. In *ex vivo* lung slices, 10 µM rottlerin resulted in relaxation of airway lumen area to 87±4% of pre-stimulation levels (n=5, $P<0.05$ vs. DMSO control). These findings correlated with increased BK channel activity (after rottlerin treatment, $V_{50}$ shifts in the hyperpolarizing direction by 73.5±13.5 mV in control cells and 71.8±14.6 mV in asthmatic cells, both n = 5 and $P<0.05$ as compared to pre-treatment) and reduced frequency of acetylcholine-induced $Ca^{2+}$ oscillations. **Conclusions:** Rottlerin reduces methacholine-induced AHR in two animal models of asthma through inhibition of airway inflammation and activation of ASM BK channels, resulting in reduced acetylcholine-induced $Ca^{2+}$ oscillations. These findings identify rottlerin as a potential therapeutic agent for asthma combining both potent anti-inflammatory and smooth muscle relaxation properties in a single compound.

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## Aberrant Adipogenesis in the Pathogenesis of Scleroderma

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Systemic sclerosis (SSc) is characterized by fibrosis in the skin and multiple organs, and is associated with aberrant TGF-beta and Wnt-beta-catenin signaling. A striking observation in SSc is the loss of subcutaneous adipose tissue (SCAT), but the underlying mechanism and significance in pathogenesis are not known. We investigated the kinetics of SCAT loss and its role in fibrosis using mouse models of scleroderma and genetic fate mapping experiments. Regulation of adipogenesis was investigated *in vitro* using multipotent mesenchymal progenitor cells and fibroblasts. The results revealed a striking loss of SCAT and replacement of adipose with fibrous tissue in the bleomycin-induced mouse model of scleroderma, which preceded the onset of dermal fibrosis. Furthermore, a decline in levels of adipogenic markers (*AdipoQ*, *Pparγ*, *aP2*) in the lesional skin preceded the increase in fibrogenic markers (*COL1A2*, *Fn-EDA*, *Periostin*). These observations led us to hypothesize that during fibrogenesis, mesenchymal progenitor cell differentiation was redirected from adipogenic towards fibrogenic fates. In vitro studies confirmed that profibrotic signals such as TGF-beta, PDGF and Wnt-beta-catenin preferentially promoted fibrogenic differentiation of mesenchymal progenitor cells *in vitro*. The biological significance of preadipocyte-fibroblast transitions in fibrogenesis was directly addressed by fate-mapping studies using adipocyte-labeled transgenic reporter mice. While adipocyte-derived cells were strictly confined to the SCAT in normal mice, they were found distributed throughout the fibrotic dermis, and expressed fibroblast markers, in mice with bleomycin-induced scleroderma. Taken together, these studies highlight a consistent association of SCAT atrophy with dermal fibrosis in SSc and in experimentally-induced scleroderma in the mouse, and suggest that adipose tissue loss may result from mesenchymal progenitor cell selection of fibrogenic fates and therefore be a primary event in fibrosis. Since pharmacological manipulation of cell fate determinations by mesenchymal progenitor cells is now feasible with drugs such as imatinib, we conclude that regulation of mesenchymal cell differentiation represents a novel therapeutic approach to fibrosis.

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## CXCL12 Production by Early Mesenchymal Progenitors is Required for Hematopoietic Stem Cell Maintenance

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Hematopoietic stem and progenitor cells (HSPCs) are a mixed population consisting of hematopoietic stem cells (HSCs) and more differentiated hematopoietic progenitors. HSPC primarily reside in the bone marrow where signals generated by non-hematopoietic stromal cells regulate their self-renewal,

proliferation, and trafficking. Understanding the physiologic regulation of HSPCs is important for improving stem cell transplants, maintaining HSPCs *ex vivo* for use in transplantation or gene therapy, and improving the treatment for hematopoietic malignancies. We hypothesized that each HSPC subset resides in a distinct niche consisting of a specific population of stromal cells and the regulatory molecules they produce. In order to test this hypothesis, we generated a novel conditional knockout mouse of the stem cell niche chemokine *Cxcl12* and selectively deleted the gene in mineralizing osteoblasts using the *osteocalcin (Oc)-Cre*, endothelial cells using the *Tie2-Cre*, perivascular reticular cells and osteoblasts using the *osterix (Osx)-Cre*, and all mesenchymal cells using the *Prx1-Cre*. We demonstrate that deletion of *Cxcl12* from mineralizing osteoblasts has no observable effect on HSPCs. In contrast, deletion of *Cxcl12* from osterix-expressing stromal cells results in a 50% reduction in bone marrow cellularity and an increased number of hematopoietic progenitors circulating in the blood. However, HSC function including long-term repopulating and quiescence activity remains normal. *Cxcl12* deletion in endothelial cells results in a modest loss of long-term repopulating activity, but all other HSC functions are intact. Strikingly, deletion of *Cxcl12* in a mesenchymal progenitor cell population results a loss of bone marrow cellularity, HSPC mobilization, and a marked loss of HSCs, long-term repopulating activity, HSC quiescence, and common myeloid progenitors. Together, these data suggest that different bone marrow stromal populations form separate niches for subsets of HSPCs. Osterix-expressing stromal cells comprise a niche that supports common myeloid progenitors and retains hematopoietic progenitors in the bone marrow, but they do not support HSCs. In contrast, mesenchymal progenitors and to a lesser extent endothelial cells are the primary support for HSCs.

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## Optimizing Anti-virulence Compounds for the Treatment of UTIs

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Uropathogenic *E. coli* (UPEC) are the major cause of urinary tract infections (UTI), one of the most common infections in women. Because there is increasing prevalence of antibiotic resistance in UPEC isolates, and because UTIs are often recurrent necessitating long-term antibiotic usage, new therapeutic strategies are needed to better treat these infections. Drugs targeting virulence factors have the potential to effectively treat bacterial infections without inducing the spread of resistant organisms, as mutations in the targeted virulence factor to decrease inhibitor action would likely also decrease the fitness of the pathogen. Here, we characterize potential therapeutics, termed pilicides that target adhesive pili. These pili are implicated in a variety of infections mediated by gram-negative bacteria. For example, UPEC use type 1 pili, which are tipped by the FimH 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 and extrusion through the outer membrane. Thus, these pili are termed chaperone usher pathway (CUP) pili. There are many types of CUP pili, and each contain adhesins at their tips that likely enable colonization of different surfaces. Each sequenced UPEC strain encodes ~10 CUP pilus operons. The most studied of these, type 1, P and S pili are associated with cystitis, pyelonephritis and neonatal meningitis, respectively. Pilicides were designed to disrupt the function of the chaperone, preventing proper pilus assembly. We demonstrate that our pilicides are effective at decreasing type 1 pilus expression and assembly. Further, we demonstrate that some pilicides are effective at preventing assembly of both type 1 pili and homologous S pili, while others have efficacy against type 1 pili alone. We also demonstrate that these pilicides alter the expression of P pili. By characterizing the effects of these potential therapeutics on type 1 pili and the other CUP pili, we begin to elucidate novel aspects of UPEC CUP pilus regulatory networks, how UPEC respond to disruptions in CUP pilus expression, and how these compounds can be effective therapeutics for infections mediated by type 1 pili and other CUP pili.

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## Histone Demethylase, KDM4C, Influences Growth of Normal and Cancer Cells

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Histone demethylases are chromatin modifiers whose biochemical activities are characterized, yet their target genes remain largely unknown. KDM4C, a member of the Jumonji family of histone demethylases, targets tri-methylated H3K9, which is associated with transcriptional repression. By treating expression levels of genes as quantitative traits in genetic analyses, we identified over 300 target genes of KDM4C. Then, by molecular studies, we showed that KDM4C regulates cell proliferation in normal and cancer cells through its target genes. We measured gene expression in B-cells from members of 45 large families, and treated gene expression levels as quantitative traits in linkage and association analyses. We found extensive individual differences in the expression level of *KDM4C* and determined that it is regulated in *cis* by variants near the 3' UTR. These *KDM4C* alleles then influence the expression levels of over 300 genes in *trans*. Using RNA interference, we depleted *KDM4C* and confirmed the regulator-target gene relationships. By chromatin immunoprecipitation, we found that KDM4C regulates its target genes by binding to their promoters. Individuals with higher *KDM4C* expression had more KDM4C at target gene promoters than individuals with lower *KDM4C* expression. Among the targets of KDM4C are genes, such as *MYC*, that play a key role in cell proliferation. By measuring several growth parameters, we showed that B- and skin cells from individuals with high *KDM4C* expression grew faster than those with lower *KDM4C* expression. In addition, we showed the *KDM4C* is expressed higher in 10 cancer types compared to corresponding normal tissues. Knockdown of *KDM4C* significantly attenuated growth of

colorectal cancer cells. In this presentation, I will show our results of how DNA sequence variants affect chromatin modifications in gene regulation, which in turn affects cellular phenotype.

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## Amniotic Fluid Fibroblasts for Production of Patient-specific Cardiac and Neuronal Tissue Prior to Birth: Implications for Congenital Heart Disease

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**Background**: Congenital heart disease (CHD) is a developmental disease in which many important steps are complete by the 6th week of human gestation. Our objective is the creation of autologous tissue using induced progenitor stem cells (iPS) prior to the child's birth as a resource for both study and repair of CHD on a patient-specific basis. **Methods**: We harvested amniotic fluid fibroblasts during the course prenatal evaluation. Source tissue was isolated with a combined mechanical and biochemical enrichment. Fibroblasts were reprogrammed to iPS cells by transduction with 5 lentiviral vectors. Following transduction, fibroblasts cultured for 2 weeks before iPS clones were identified and characterized. iPS cells were differentiated *in vitro* to cardiac and neuronal tissue using a directed differentiation protocols and analyzed by RTqPCR and immunohistochemical markers on a strict time line. **Results**: We collected amniotic fluid (n=9) at gestational age 19-25 weeks and created stable cell lines (n=91). After reprogramming, iPS clones were characterized and differentiated down the cardiac and neuronal lineages. Successful directed differentiation to cardiac and neuronal tissue was completed by gestational age 36, one month prior to birth. **Conclusions**: We have successfully produced iPS-derived patient specific tissue from patients with structural heart disease. Reprogramming efficiencies are high and likely due to young age. This innovative technique maintains the general genetic background to facilitate study of the developmental basis of human disease in a patient-specific manner. Importantly, we were able to do so from amniotic fluid cells prior to birth. This experiment establishes the scientific principals for production of autologous tissue prior to birth.

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## Mechanisms of Therapeutic Hypothermia in Cardiac Arrest: Regulation of Autophagy by Xytoplasmic p53 in Cardiomyocytes Following Oxidative Stress

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**Introduction:** Cardiovascular disease results in over 300,00 deaths per year in the US. 90% of patients die within the first 24 hours of a cardiac arrest event with oxidative injury from ischemia-reperfusion after cardiac arrest as a leading cause of mortality. Currently therapeutic hypothermia (TH) is the only treatment known to improve mortality. It is hypothesized that the protective effects of therapeutic hypothermia on cardiomyocytes (CMC) during cardiac arrest include inhibition of cytoplasmic p53. Cytoplasmic p53 is believed to normally play a dual role in the heart promoting apoptosis and inhibiting autophagy. **Methods:** HL-1 cells were treated with combinations of Pifthrin-α (Pif - a cytoplasmic p53 inhibitor), Bafliomycin-A1 (Baf -an inhibitor of autophagosome-lysosome fusion, blocking degradation of autophagosomes used to enhance the signal of autophagy), and deferoxamine (DFO – a hypoxia mimic that induces autophagy). Cells were then lysed 18 hours later and run on a western blot that was probed for levels of LC3I and LC3II. LC3 converts from LC3I to LC3II during autophagosome formation The LC3II:LC3I ratio was used as a marker of autophagy. **Results:** In these studies we found that HL-1 cells treated with Baf showed increased levels of the ratio in both control and DFO samples. HL-1 cells treated with Pif did not show an increase in autophagy as compared to control, however, cells treated with Pif and DFO showed a significant increase in the ratio as compared to just DFO. Finally, when treated with Baf and Pif cells showed an increase in the ratio as compared to the control in both control and DFO conditions. **Conclusions:** Together, these findings suggest that when cytoplasmic p53 normally inhibits autophagy following stress to the cells. When treated with Pif cells that were subjected to DFO showed significantly higher amounts of autophagy but when hypoxia was not mimicked, as in the control condition, treatment with Pif did not increase autophagy. Additionally, treatment with Baf can be concluded to increase the LC3II:LC3I ratio as a marker for more accurate measures of autophagic flux. Funding in part: NIH 5-R01-HL084643, NIH funded summer research program at Pritzker School of Medicine.

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## Hdac3 Plays a Critical Role in Cardiac Development and Function

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Class I and II histone deacetylases (HDACs) are necessary for proper cardiac development and function, and HDAC inhibitors have demonstrated therapeutic potential in cardiac hypertrophy and other disorders. Recent studies have shown that Hdac3 is critically important in regulating the cardiac response to high fat diet, however little is known about the role of Hdac3 in early cardiac development and its function in cardiac progenitor cells. We have genetically deleted *Hdac3* in several cardiac progenitor populations spanning embryonic days 6.5 to 9.5, and found that loss of *Hdac3* at embryonic day eight or earlier leads to the formation of hearts with thin myocardium and embryonic lethality. Interestingly, *ex vivo* experiments with explanted cardiomyocytes reveal that e9.5 *Hdac3*-deficient cells have similar gene expression profiles as e14.5 wild-type cardiomyocytes. Furthermore, deletion of *Hdac3* in differentiating mouse embryonic stem cells induces early differentiation of multipotent cardiac progenitor cells towards the cardiomyocyte lineage. Taken together, these data lead us to hypothesize that loss of *Hdac3* in the developing heart causes precocious differentiation of cardiac progenitor cells, depleting the pool of progenitors and resulting in a thin myocardium.

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### Chronic Sleep Disruption Accelerates TC1 Cell Tumor Growth and Invasiveness via TLR-4 Signaling and Recruitment of Tumor-Associated Macrophages

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Disrupted sleep (SD) is a highly prevalent condition that is associated with increased cancer mortality. Here we explore whether SD alters tumor growth via TLR-4 pathways and recruitment and activation of TAM in the tumor milieu. C57/b6, TLR4-/-, MYD88-/- and TRIF-/- male mice were exposed to 35 days of SD using a custom-designed apparatus along with 110 controls (SC) matched. On day 7, all groups were challenged with 1 *10^5 TC1 tumor cells injected SC into the right lower flank; tumor size was monitored using calipers till day 28 after injection, at which time tumors were enucleated, weighed, and subjected to FACS for TAM (CD45+ CD11b+ F4/80+), and IHC. To assess differences in local tumor invasiveness, another set of C57/b6 mice was SC injected with TC1 cells into the right thigh followed by histological assessments and MMP-9 imaging. Accelerated tumor size growth emerged in SD-C57/b6, with significant differences emerging at day 23 of injection ($p< 0.007$), as well as tumor weight at day 28 (SD-57/b6: 1.955± 0.680 g *vs.* SC-C57/b6: 1.046±0.479 g; $p<0.001$). Moreover, increased invasiveness was apparent in SD- C57/B6 tumors by both IHC and MMP-9 imaging ($p<0.01$). Increased TAM counts occurred in SD-C57/b6 tumors ($p<0.02$), and were distributed in close proximity to the tumor capsule, as compared to preferential tumor core location in SC-C57/b6 mice. TAM from SD-exposed mice expressed high levels of TLR4 m RNA and protein, and their numbers were reduced in SD-TLR4 -/- tumors ($p<0.001$). Significant reductions in tumor size and weight occurred not only in SC-TLR4-/- mice, but the accelerated growth induced by SD was completely abrogated in SD-TLR4-/- mice ($p<0.001$). Interestingly, although tumors enucleated from SD-MYD88-/- and SD-TRIF-/- mice showed reductions in tumor weight compared to SD-C57/b6 ($p< 0.03$, $p<0.01$ respectively), tumors were larger than in SD-TLR4-/- mice Chronic SD, such as occurs in multiple sleep disorders, induces accelerated tumor growth, expansion and invasiveness in a solid tumor model. The increased numbers and differential tumor location of TAM and associated MMP-9 expression suggest that SD-mediated effects on TAM polarity and function may underlie the increased invasive and aggressive tumorigenesis in SD. Moreover, the significant reductions in tumor growth and TAM numbers in the SD-TLR4 -/- mice, and the intermediate effects found in SD-MYD88-/- and SD-TRIF-/- mice further indicate that TLR4 signaling pathways operate as major contributors to the SD mediated effects on tumor progression, TAM polarity and function.

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### A Human Cellular Model of Friedreich Ataxia Indicates a Major Role for the DNA Mismatch Repair Protein PMS2 in Trinucleotide Repeat Expansion

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Over three dozen neurodegenerative diseases including Huntington's disease, Myotonic Dystrophy, and Friedreich Ataxia (FRDA) are caused by trinucleotide repeat expansions. Evidence suggests that the number of triplet repeats directly correlates to disease severity. Consequently, from a clinical perspective slowing the progression of repeat expansion is of particular interest. FRDA is a fatal, autosomal recessive disease that is clinically characterized by progressively worsening limb and gait ataxia; it is caused by a GAA•TTC triplet repeat in the first intron of the frataxin gene. To study the mechanism of this disease, our lab has developed a human cellular model of FRDA that recapitulates the incremental, continuous expansion found in the post-mitotic neurons of affected patients. This model's accelerated time course enables us to rapidly identify potential targets that might slow or halt the rate of expansion and thus disease progression in FRDA. Using this model, we have demonstrated that the heterodimer MutSβ, a component of the DNA mismatch repair pathway (MMR), plays a significant role in the expansion rate of GAA•TTC repeats. MutSβ is a recognition complex composed of MutS Homologue 2 (MSH2) and MSH3. In our model system, depletion of either MSH2 or MSH3 significantly slows the rate of GAA•TTC expansion. While MutS complexes are necessary for identification of DNA mismatches, the recruitment of MutL incision complexes are necessary for processing them. Therefore, we postulated that if MutSβ is involved in GAA•TTC expansion, then MutL complexes must also contribute to the expansion process in FRDA. Consistent with this hypothesis, our results demonstrate that depletion of the protein Post Meiotic Segregation Increased 2 (PMS2), an essential component of the MutLα complex, significantly increases the rate of GAA•TTC repeat expansion in our human cellular model. These results further emphasize that the MMR pathway is a critical target for understanding the mechanism of trinucleotide repeat expansion in FRDA.

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### Characterization of Synaptosomal Mitochondria Derived From Brain Regions Containing Central Dopamine Neurons in Parkin Deficient Mice

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Parkinson's disease (PD) is the second most common neurodegenerative disorder that causes tremor, rigidity, bradykinesia, and postural instability. Administration of L-DOPA, dopamine (DA) agonists, and deep-brain stimulation therapies can alleviate motor symptoms. The hallmark pathology of PD is

progressive degeneration of nigrostriatal (NS) DA neurons, which underlies the motor symptoms of PD. However, the molecular mechanism of DA neurodegenerative is not known. While there is severe loss of midbrain NSDA neurons, the hypothalamic tuberoinfundibular (TI) DA neurons remain intact. A similar pattern of susceptibility can be seen in these DA neuronal populations following exposure to mitochondrial Complex I inhibition *in vivo* and differential expression of parkin was observed. Parkin is a 52Kda cytosolic protein originally identified by linkage analysis in autosomal recessive juvenile parkinsonism. *In vitro* studies reveal that parkin plays a significant role in quality control of mitochondria by tagging damaged mitochondria for mitophagy without inducing ROS or apoptosis. Here, we identified the first *in vivo* evidence of parkin deficient mitochondrial dysfunction. Mitochondrial bioenergetics, membrane potential, mass, and the morphology were determined in synaptosomes isolated from brain regions containing NSDA and TIDA neurons of wild-type (WT) and homozygous parkin knockout (KO) mice. Basal respiration rates were similar in both regions, but spare and maximum respiratory capacities were significantly lower in both striatum (ST) and mediobasal hypothalamic (MBH) synaptosomes derived parkin KO mice as compared with WT controls. In addition, flow cytometric analysis revealed that less mitochondrial mass was present in ST and MBH derived synaptosomes obtained from parkin KO than WT mice. Furthermore, transmission electron microscopy revealed that WT mice had much higher Fleming mitochondrial function score and more intact morphology compared to parkin KO mice. These results suggest that impaired synaptosomal mitochondrial function may be due to decreased numbers of viable mitochondria in parkin deficient mice, possibly due to loss of parkin-mediated mitochondrial quality control.

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## The Most Frequently Mutated Mitogenic Pathway in HNSCC: The PI3K/AKT Pathway

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Genomic heterogeneity in HNSCC presents a major obstacle to broadly effective therapy. We and others recently reported mutational profiles of over 100 HNSCC tumors. To date, translational gaps between genomic findings and patient treatment selection in HNSCC exists. Mitogenic pathways are vital to cancer development and progression. Mutations in mitogenic pathways have been shown to result in pathway activation, increased proliferation, and enhanced sensitivity to agents targeting the activated pathway. We performed a bioinformatics analysis of 151 HNSCC to examine the mutational profiles of major mitogenic pathways previously shown to be important in HNSCC, including MAPK, JAK/STAT and PI3K/AKT, and found that the PI3K/AKT pathway is the most frequently mutated mitogenic pathway (30.46% cases; 46/151 tumors) followed by JAK/STAT and MAPK, (9.27% cases; 14/151 tumors) and (7.95% cases; 12/151 tumors), respectively. HNSCC tumors with mutations in PI3K/AKT pathway harbored 2.3 times more non-synonymous mutations (165.50 ± 24.08 vs 72.05 ± 6.63 mutations, P<0.0001) and twice as many cancer gene mutations than WT PI3K/AKT pathway tumors (7.15 ± 0.75 vs 3.56 ± 0.29 mutations, P<0.0001) including multiple mutational events of genes in the PI3K/AKT pathway. Interestingly, in 3 out of 45 HPV(+) HNSCC tumors, *PIK3CA* was the only cancer gene found to be mutated, suggesting that the PI3K/AKT pathway alone may be sufficient to drive some HPV(+) HNSCC. The observed frequency of *PIK3CA* mutation in our HNSCC cohort (12.58%; 19 mutations total) is higher than that reported previously in other smoking-related cancers such as lung cancer and esophageal cancer, where the respective *PIK3CA* mutation rates are no greater than 3-5%. Other components of the PI3K/AKT pathway were mutated in <2-3.97% of tumors. Downstream effectors, including *PDK1, AKT1* were not mutated, while *AKT2* and *mTOR* were mutated in just 1.29% of tumors. *PIK3CA* and the PI3K/AKT pathway are currently targetable in human cancers, with several agents in various stages of clinical development. The growth of HNSCC xenografts derived from a cell line with a *PIK3CA*(H1047R) mutation, treated with the mTOR/PI3K inhibitor BEZ-235, was significantly inhibited. *PIK3CA*, and potentially other PI3K/AKT pathway, mutations may serve as predictive biomarkers in HNSCC to guide treatment selection.

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## Characterization of the Genetic Etiology of von Willebrand Factor and Coagulation Factor VIII Levels in a Multigenerational Amish Cohort

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Low levels of VWF and FVIII cause clinically significant bleeding while high levels are associated with thrombosis. Closed populations have proven valuable in elucidating genes of Mendelian traits and are becoming more recognized in the study of complex and quantitative traits due in part to a more homogenous environmental component. We characterized a multigenerational Amish family with von Willebrand disease caused by a single autosomal-dominant mutation resulting in a R1374C substitution and demonstrated variability between and reproducibility within individuals of coagulation factor VIII (FVIII:C) and von Willebrand factor antigen (VWF:Ag) and ristocetin cofactor (VWF:RCo, functional assay) levels. Analysis in SOLAR estimated a heritability of 0.301 for VWF:Ag, 0.386 for VWF:RCo, and 0.280 for FVIII:C. Genome-wide quantitative trait locus (QTL) linkage analysis in 384 individuals identified QTLs on chromosome 9q33 (ABO), 12p13 (VWF), and 16q11 (novel locus) for VWF:Ag and VWF:RCo with linkage on 12p13 for FVIII:C. Suggestive linkage for VWF:Ag was identified to 6p22, replicating findings from the Framingham Heart and GAIT studies. *ABO*, the best-characterized QTL of *VWF*, explained 8, 12, and 7% of the variability of VWF:Ag, VWF:RCo, and FVIII:C , respectively, and type O blood is overrepresented. Of 94 HapMap tagging SNPs genotyped through *VWF*, rs7964554 (intron 4) demonstrated the most significant association with VWF:Ag and VWF:RCo. SNPs near the functional domains of VWF were associated with VWF:RCo. Of the 9 SNPs identified in the Amish cohort evaluated to date, 6

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SNP associations were replicated in an unrelated healthy cohort and 4 SNPs were replicated in the Genes and Blood Clotting Study sibling cohort. Candidate QTLs on 6p22 and 16q11 are currently being fine-mapped. The recent CHARGE consortium meta-analysis reported 8 chromosomal regions associated with VWF levels, 5 of which are also associated with FVIII:C, and the most significant intragenic SNP for each region. We genotyped 10 of 13 SNPs covering all 8 genes in 448 members of our Amish cohort and replicated significant associations for 6 SNPs in *VWF*, *ABO*, *STXBP5*, *SCARA5*, *STAB2*, and *TC2N*. Exonic sequencing of *STXBP5* and *STAB2* in 48 samples identified only common variants in dbSNP. All FVIII QTLs overlap with VWF QTLs suggesting FVIII levels are mediated by VWF in healthy individuals.

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## Risk of Breast Cancer With Papillary Lesions

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**Background:** Breast cancer is the second most commonly occurring cancer in women in the U.S. Prompt diagnosis and treatment of breast cancer is critical for optimal prognosis. There are currently no firm guidelines for excision with a finding of a papillary lesion on core biopsy. **Purpose:** The purpose of this study was to evaluate whether the diagnosis of a papillary lesion on core biopsy was a risk factor for breast cancer. **Methods:** The pathology records at our institution were queried for the diagnosis of a papillary lesion on core biopsy between the years 1999 and 2011. Electronic and paper charts were then retrospectively reviewed for demographic data, clinical and imaging findings and breast cancer risk factors. Pathology reports of those patients who underwent excision were reviewed. Chi-squared, Fisher's Exact and ANOVA analysis were performed to identify associations between clinically significant variables and final pathology. Follow-up at our institution with any physician was tracked and the Social Security Death Index was searched for possible patient deaths. **Results:** There were 147 core biopsies with a diagnosis of a papillary lesion in 132 patients. Fifty-four patients with a concurrent diagnosis of breast cancer (20 ductal or lobular infiltrating carcinoma, 18 DCIS, and 6 papillary carcinoma) were excluded from the study. Fifty-two (55.9%) of the remaining 93 patients underwent excision. Other high risk lesions (12 ADH, 2 ALH, 1 LCIS) were identified in 15 patients. Breast cancer was diagnosed in 10 (19.2 %) patients. Calcifications seen on mammogram and post-menopausal status were found to be independent predictors of breast cancer on excision. Six patients developed breast cancer more than one year after excision (3 contralateral, 3 ipsilateral). Follow-up beyond 1 year was available for 30 (56%) patients who did not undergo excision initially. Five (16.7%) of those patients subsequently developed breast cancer (2 contralateral, 3 ipsilateral). **Conclusions:** Calcifications on mammogram and post-menopausal status are predictive factors for cancer for a patient with a papillary lesion. Since 19.6% of patients with papilloma on biopsy were found to have cancer after excision, it is recommended that all patients with papillary lesions undergo excision. Larger numbers in future studies would allow for more robust interpretation of the data on predictive factors.

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## Nitric Oxide and Carbon Monoxide Decrease Transforming Growth Factor Beta Signaling Through a Dynamin-2 Dependent Process

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**Background:** Transforming growth factor-β (TGF-β) is a profibrotic growth factor that participates in vascular structure and function. Signal propagation is responsible for deposition of extracellular matrix proteins and promotion of vascular fibrosis. Previous studies from our lab demonstrated that nitric oxide (NO) mitigates the deleterious effects of TGF-β. Studies from other laboratories using bladder endothelial cells and human embryonic kidney (HEK) cell lines indicated that NO activates dynamin-2, a GTPase that is integrally involved in both clathrin- and caveolae-mediated endocytic vesicle formation. In addition to the role of NO in blood vessels, there is a growing appreciation for the role of carbon monoxide (CO) as a highly diffusible, bioactive signaling molecule. We hypothesized that both NO and CO increase endocytosis of the TGF-β type I receptor (TBRI) in vascular smooth muscle cells (VSMC) through activation of dynamin-2, potentially shielding the cells from the effects of circulating TGF-β. **Methods:** Primary cultures of VSMC from Sprague-Dawley rats were treated with NOR3 (a NO chemical donor), CORM2 (a CO chemical donor), ODQ (an inhibitor of soluble guanylyl cyclase), or vehicle. In some experiments, cells were pretreated with dynamin-2 siRNA or control for 48 hours. Dynamin-2, SMAD signaling proteins, calponin, and SM22α were detected using commercially available antibodies. Surface expression of TBRI was detected with fluorescence-activated cell sorting (FACS). **Results:** Physiological levels of NO and CO stimulated in a dose-dependent fashion dynamin-2 multimerization indicating activation of dynamin-2. NO and CO stimulated a time- and dose-dependent endocytosis of TBR1 in a guanylyl cyclase-independent fashion. Cells pretreated with dynamin-2 siRNA did not demonstrate a NO- or CO-stimulated decrease in surface expression of TBRI. Compared to control experiments, NO and CO decreased Smad2 phosphorylation and the production of calponin and SM22α by VSMC. **Conclusions:** NO and CO diminished the effects of TGF-β in VSMC by decreasing TBRI surface expression through a dynamin-2 dependent process. The findings help explain an important mechanism by which NO and CO protect the vasculature by decreasing surface expression of the TGF-β type I receptor and, therefore, decreasing the cellular response to the profibrotic growth factor, TGF-β.

# Poster Abstracts

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### Oncogenic c-Myc Disrupts Circadian Rhythm

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Circadian rhythms are regulated by feedback loops of core clock genes with a periodicity around 24 hours. Disrupted circadian rhythm results in a variety of pathological state, including cancer. However, there is no basis of how circadian rhythm is perturbed in cancer. Oncogenic Myc is a transcription factor that is widely expressed in many cancers. Although the role of Myc in circadian rhythm is unknown, it may alter circadian rhythm by binding promoters of clock-controlled genes at E-box [CACGTG] sites, which are also used by the central circadian regulator Clock-Bmal1. Myc may also change the NAD+ level in the cells through upregulation of NAMPT, which in turn affects the activity of circadian regulator Sirt1. Here we show in hepatocellular carcinoma and Burkitt's lymphoma cells that overexpress ectopic Myc, Myc specifically upregulates nuclear receptor Rev-erbα, a negative regulator of Bmal1 transcription. Inhibition of either Rev-erbαor NAMPT alters circadian gene expression. Overexpressed Myc also dramatically disrupts circadian oscillations. This work demonstrates that Myc alters circadian oscillation through upregulation of Rev-erbα and NAMPT. These findings provide insights into the circadian disruption in Myc-driven cancer and may ultimately lead us to uncover a therapeutic window hidden in the oscillation of metabolism between cancer cells and normal cells.

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### BUD31 is a Novel Therapeutic Target in c-Myc-driven Breast Cancer

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**Introduction**: Amplification and/or hyperactivation of c-Myc (Myc) occurs in 20-40% of human malignancies and confers poor prognoses in many cancer subtypes. While direct targeting of Myc has been unsuccessful to date, we and others have searched for the stress support pathways required to tolerate aberrant Myc activation. Such pathways represent ideal therapeutic targets because cancer cells become hyper-dependent on them for survival whereas normal cells and tissues do not. Using a genome-wide RNA interference (RNAi) screen, we searched for genes required to tolerate aberrant Myc activation (Kessler et al., Science 2012). We discovered that cells are strongly dependent on the spliceosomal protein BUD31, which is involved in pre-mRNA intron removal in yeast but is poorly understood in humans. **Methods**: *BUD31* was validated as a Myc-synthetic lethal gene using a conditional-Myc/conditional-*BUD31* cell system we developed. We assessed the effect of inactivating Bud31 and its associated partners in the spliceosome on a panel of Myc-driven breast tumor models using newly developed inducible RNAi technologies and pharmacologic inhibitors of BUD31 sub-complex. **Results**: Our validation studies show that BUD31 is critical for tolerating Myc hyperactivation. BUD31 inactivation impairs cancer cell viability and *in vivo* tumor progression in Myc-driven breast cancers. Using mass spectrometry studies we identified BUD31-interacting proteins, and we validated their synthetic lethal relationship with Myc. Putative pharmacologic inhibitors of BUD31-associated spliceosomal complexes also selectively impair the survival of Myc-driven breast cancer cells *in vitro*. **Conclusion**: *BUD31*, a top candidate from a genome-wide Myc-synthetic lethal screen, is necessary for breast cancer cells to tolerate aberrant Myc hyperactivation *in vitro* and *in vivo*. Components of BUD31-related complexes are also essential to tolerate oncogenic Myc, and small molecule splicing inhibitors of BUD31 complexes demonstrate selective targeting of cells with hyperactive Myc. By understanding how BUD31 and its associated complex play a role in coping with Myc stresses, we will identify potential targets for therapeutics that can be specifically applied to aggressive Myc-hyperactive breast cancers. Furthermore, insight into stress support networks may be applied to other Myc-driven malignancies, which will make a substantial impact on the treatment of cancer patients.

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### Neural and Genetic Basis of Circadian Regulation of Mammalian Glucose Homeostasis

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The circadian gene system is encoded by a conserved transcription-translation feedback loop that coordinates behavioral and energetic cycles in mammals. The neural circuit encoding the clock is organized hierarchically; neurons localized to the suprachiasmatic nucleus (SCN) superior to the optic chiasm function as the master pacemaker. SCN neurons form reciprocal connections with cells of the fore-, mid- and hind-brain involved in glucose homeostasis. Disruption of circadian rhythms in humans and mice results in impaired glucose metabolism, however the underlying function of pacemaker and extra-pacemaker neurons in glucose homeostasis remains unclear. Importantly, core clock transcription factors are also expressed within many peripheral tissues involved in glucose metabolism, and in pancreatic islets, clock gene ablation leads to hypoinsulinemia and diabetes mellitus. What is still not understood is how synchrony between and amongst central and peripheral clocks contributes to stable rhythms of glucose metabolism across the day and night. Here, we present initial results using the Cre-LoxP strategy to dissect the function of the circadian system within brain on glucose oscillation, glucose tolerance, and insulin sensitivity. Brain clock mutant mice are overtly entrained

to light, however, these animals display blunted and abnormal glucose rhythms, impaired glucose tolerance, and insulin resistance. Clock gene expression within brain is therefore critical to mammalian glucose homeostasis, revealing a genetic linkage between neuronal circuits regulating behavior and metabolism.

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## Fc-Region Specific IgG Conjugation Onto Nanoparticles and ELISA Microplates

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**Introduction:** Labeling nanoparticles with targeting antibody is crucial for diagnostic and therapeutic applications. However, most antibody conjugation techniques suffer from the lack of orientation specificity, causing suboptimal epitope binding due to steric hindrance. Here, we utilize Expressed Protein Ligation (EPL) and click chemistry to achieve site-specific conjugations of native antibodies. We recombinantly expressed an antibody-binding protein that can be conjugated to nanoparticles in a site-specific manner. By incubating antibodies with these pre-prepared nanoparticles, versatile, site-specific, and efficient antibody labeling is achieved. Additionally, we also explored covalent conjugation by incorporating an UV active moiety into the antibody-binding protein, as well as using this technique for antibody conjugation onto microplate for ELISA applications. This method gives improved detection sensitivity and versatility since all IgGs have optimally oriented Fab and that nearly all IgGs are suitable. **Results and Discussion:** Recombinant IgG-binding domain of Protein A was successfully expressed in E coli using an EPL-capable plasmid. The fusion protein was purified using chitin bead column. FITC-labeled peptides with a click-capable azide moiety (Azido Fluorescent Peptide-AzFP) were synthesized commercially. Combining the fusion protein with AzFP allowed Protein A to be ligated to AzFP via EPL, as confirmed by SDS-PAGE. Next, by incubating antibody with Protein A-AzFP, the IgG Fc region was bound with optimal affinity and orientation, as confirmed with dot blotting and native PAGE. This antibody-Protein A-AzFP construct can then be coupled onto any amine coated nanoparticles (ie. SPIO) via an efficient click reaction. Alternatively, Protein A-AzFP can first be coupled onto nanoparticles, generating versatile pre-prepared nanoparticles that can be stored and then later be efficiently labeled with any antibody. Labeling is confirmed by fluorescent and MR imaging. Currently, we are also exploring covalent conjugation of IgG by engineering Protein A to contain a photoactivatable moiety, such as photo-methionine, in its binding site, which then forms a covalent bond to the IgG upon UV irradiation. Additionally, we have also explored using this system to conjugate capturing IgG onto ELISA microplates in an orientation-specific manner.

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## Heme Oxygenase-1 Expression Protects the Myocardium from Cre-induced Toxicity

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The protective effect of heme oxygenase-1 (HO-1) expression in cardiovascular diseases including ischemia-reperfusion injury and allograft rejection has been previously demonstrated using transgenic animal models that constitutively overexpression HO-1 in the heart. However, the temporal requirements for protection by HO-1 induction relative to injury have not been investigated, but are essential to employ HO-1 as a therapeutic strategy in human cardiovascular disease states. Therefore, we generated mice with cardiac-specific, tamoxifen (TAM)-inducible overexpression of a human HO-1 (hHO-1) transgene (MHC-HO-1 mice) by breeding a mouse with cardiac-specific expression of a TAM-inducible Cre recombinase (MHC-Cre mice) with a mouse containing an hHO-1 transgene preceded by a floxed stop signal (CBA-flox mice). In MHC-HO-1 mice, TAM administration (40 mg/kg body weight on two consecutive days) causes significant overexpression of the hHO-1 gene and enzymatically active protein at day 1, with maximal induction occurring at day 3. Although MHC-Cre mice are commonly used for gene manipulation studies in cardiovascular research, it was recently shown that TAM-inducible Cre causes acute cardiac toxicity in these mice. Here, we demonstrate that cardiac-specific overexpression of hHO-1 prevents the Cre-induced toxicity. Following TAM administration, MHC-Cre mice develop acute depression of systolic function and dilated cardiomyopathy that results in >80% mortality three days after TAM administration. Examination of cardiac sections from MHC-Cre mice revealed severe transmural inflammation and diffuse cardiomyocyte necrosis. Analysis of the inflammatory cells in the heart by flow cytometry revealed significant infiltration of neutrophils (18-fold) in MHC-Cre mice treated with TAM, while the mononuclear phagocyte population is unchanged. In MHC-HO-1 mice, cardiac hHO-1 overexpression prevents mortality and depression of cardiac function following TAM administration. In the heart of MHC-HO-1 mice treated with TAM there is no evidence of cardiomyocyte necrosis and no significant increase in the number of neutrophils, relative to vehicle treated controls. Our results demonstrate that HO-1 induction is sufficient to prevent cardiac toxicity in mice with TAM-inducible Cre recombinase expression by protecting the heart from necrosis and neutrophil infiltration. These findings are important because MHC-Cre mice are widely used in cardiovascular research despite the limitations imposed by Cre-induced cardiac toxicity and also because inflammation is an important pathological component of many human cardiovascular diseases.

# Poster Abstracts

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### Deletion of Myostatin Improves Vascular β-adrenergic Function in Mice

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Exercise increases muscle mass but the cardiovascular benefits of increased muscle mass are controversial. The deletion of growth factor myostatin (Ms) increases muscle mass but the impact on cardiovascular function is unknown. The current study determined if cardiac vascular function was affected by myostatin deletion. On an ICR background, lean and Ms-null mice were similar in body weight but Ms-null mice displayed significantly lower visceral fat and plasma leptin levels. Fasting glucose and lipids were comparable between groups. Blood pressure and heart rate assessed by radiotelemetry were similar in control and Ms-null mice. While heart mass, myocyte size and end diastolic diameter were similar between groups, deletion of Ms increased ejection fraction by 35% (27.9 ±1.0 vs. 37.4±1.9%). Ejection fraction in Ms-null mice was increased during β-adrenergic stimulation (isoproteronol) and blockade (propranolol) in a parallel fashion. In isolated aortae, vasoconstriction to a -adrenergic stimulation (phenylephrine) was modestly reduced (126 ±11 vs. 101±5% of KCl, $p<0.05$). Vasodilation to isoproteronol was markedly increased (22 ±4 vs. 51±9%, $p<0.05$). Inhibition of nitric oxide (NO)-production significantly reduced the isoproteronol response in both groups and non-adrenergic NO-mediated dilation was similar. Activation of $\beta_3$ receptors failed to elicit dilation in either group. Taken together, these data indicate that deletion of myostatin does not affect mean arterial pressure in mice, increases fractional shortening at all levels of inotropy, reduces aortic a-adrenergic constriction and increases aortic β-adrenergic vasodilation. Overall, an increase in muscle mass appears to favor improved perfusion to catecholamine activated states. The cellular mechanism remains to be determined.

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### Cellular Interconversion is Conserved Across Multiple Adult Progenitor Populations

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Multiple adult tissues, such as the intestine, skin, and bone marrow, have been thought to possess two distinct stem cell populations, quiescent and cycling. However, the identity and characteristics of these populations have been the subject of controversy. Our work demonstrates that *Hopx*, an atypical member of the homeobox family of proteins, is a specific marker of quiescent intestinal stem cells at the +4 position. Using novel genetic models, our data provide evidence that *Hopx*$^+$ cells contribute to all of the differentiated lineages of the intestine. Furthermore, *Hopx*$^+$ cells can give rise to cycling, *Lgr5*$^+$ cells at the intestinal crypt base, cells that also possess the capacity to give rise to *Hopx*$^+$ cells as well as the entire intestinal epithelium. These findings suggest a bidirectional lineage relationship between these progenitor populations. *Hopx* is also a specific marker of label-retaining hair follicle stem cells and can give rise to cycling, *Lgr5*$^+$ cells over time. Previous studies have shown that *Lgr5*$^+$ cells in the skin can give rise to quiescent stem cells. Finally, our most recent work suggests that *Hopx* is expressed by Type I pneumocytes in the adult lung but not by Type II pneumocytes. However, upon injury, *Hopx*$^+$ cells can give rise to Type II pneumocytes. Taken together, these studies across multiple tissues suggest that cellular interconversion may be a mechanism employed by multiple adult progenitor populations to maintain homeostasis.

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### Cancer Cell Proliferation is Inhibited by Specific Modulation Frequencies

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**Purpose:** Hepatocellular carcinoma (HCC) incidence in the US is dramatically increasing. Five-year survival remains 3-5%, demonstrating urgent need for additional therapies. Intrabuccal administration of amplitude modulated electromagnetic fields (RF EMF) is a novel, minimally invasive treatment modality which results in whole body absorption of very low levels of RF EMF. Clinical studies show that this treatment approach elicits therapeutic responses in patients with hepatocellular carcinoma and breast cancer. Using an *in vitro* exposure system replicating the levels of exposure achieved in humans, we have described a phenotype in HCC cells following RF EMF exposure that included proliferative inhibition, modulation of gene expression, and disruption of the mitotic spindle. This phenotype was specific for HCC cells exposed to HCC-specific RF EMF at exposure levels ranging from 0.03 to 0.4 W/kg. **Methods:** HCC cells were exposed to 27.12 MHz electromagnetic fields modulated at specific frequencies in the audio range, previously identified in HCC patients. MicroRNA arrays compared exposed and control groups of HCC cells, with microRNA validation followed by Western blot of target genes and proteins. HCC xenografts were injected subcutaneously in NOD SCID mice. Following palpable tumor establishment, mice were exposed to HCC-specific RF EMF at a specific absorption rate of 0.4 W/kg, euthanized following excessive tumor burden, and evaluated by immunohistochemistry. **Results:** We identified increased levels of miRNAs targeting proteins belonging to the PI3K pathway, specifically IP3/DAG signaling and intracellular calcium release. This pathway is frequently disrupted in HCC and breast cancer, making it an excellent candidate for modulation by RF EMF; furthermore, downstream effects include: cell cycle progression, proliferation, inhibition of apoptosis, and cell migration. We observed tumor shrinkage in mice exposed to HCC-specific modulation frequencies and residual xenograft tumor cells were infiltrated with fibrous tissue and showed significantly decreased proliferation and increased apoptosis. There was no evidence of altered cell proliferation or fibrosis in other organs. **Conclusion:** These findings are the first evidence of the efficacy and safety of RF EMF in HCC using a subcutaneous xenograft

model and uncover a novel mechanism that controls cancer cell growth *in vivo* at specific modulation frequencies, possibly through modulation of PI3K signaling and downstream release of intracellular calcium.

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## Late Effects on the Megakaryocyte Lineage from Internal and External Ionizing Radiation

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**Background:** All blood cells are derived from hematopoietic stem cells (HSC) that differentiate into lineage-committed progenitors that in turn mature into morphologically identifiable precursors. It has long been known that exposure to whole body irradiation (WBI), can disrupt this system, resulting in life-threatening cytopenias, particularly thrombocytopenia. A nuclear accident or attack will result in both external radiation exposure and internal contamination through inhalation and ingestion of radioactive particles. Little is known about the late radiosensitivity of the megakaryocyte lineage to external versus internal radiation exposure. **Objectives:** In this investigation we set out to determine the radiosensitivity of the megakaryocyte lineage in-vivo of mice exposed to sub lethal total body irradiation (TBI), internal Cesium137 contamination, or a combination of the two. The goal was to determine the late effects of a one-time external TBI exposure versus an internal radiation exposure on megakaryocytes precursors. **Methods:** Mice were exposed to 0Gy, 2.5Gy, 6 Gy TBI, with or without 100uCi of internal $Cs^{137}$ delivered by intraperitoneal injection. Slides were stained and underwent multispectral analysis with Image Pro Analyzer 7.0 and an algorithm created by our Imaging Corp. megakaryocyte precursors were hand tallied. **Results:** We found that in mouse Diaphyses the megakaryocyte precursor number were significantly decreased in only the 6Gy+100uCi condition at 12 weeks, but were significantly decreased in the 2.5Gy, 100uCi and 6Gy + 100uCi conditions at 26 weeks, but not the 6Gy alone. The findings were more dramatic in mouse Metaphyses where the megakaryocyte precursor counts were 3.22Sq/mm at 12 weeks post 2.5Gy and 0.48Sq/mm at 26 weeks. **(**$p=7.94x10^{-7}$) **Conclusions:** Megakaryocyte precursors were recovered from all but the harshest injury at 12 weeks post insult. Internal radiation and low dose radiation were significantly more toxic to Megakaryocyte precursors at 26 weeks than an initial dose of near lethal TBI (6Gy). There also appears to be a differential effect in HSC compartments between Diaphyses and Metaphyses. We hope that ultimately this research may allow for a better understanding of factors that could mitigate the effects of ionizing radiation on megakaryocyte precursors and ultimately platelet production and hemostasis.

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## Akt and mTOR Pathways Differentially Regulate Natural and Inducible IL-17 Producing CD4+ T Cells

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Interleukin-17 (IL-17) producing CD4+ T helper (Th) cells, Th17 cells, are essential for immune responses against extracellular pathogens. Dysregulation of Th17 cells, meanwhile, leads to the pathogenesis of many inflammatory and autoimmune disorders, and modulating the IL-17 axis in these diseases has been met with therapeutic success. Recent studies have revealed that two distinct populations of Th17 cells exist. Inducible Th17 (iTh17) cells differentiate from naïve $CD4^+$ T cells in response to antigenic stimulation in the presence of an appropriate cytokine environment in the periphery, most notably the intestine dependent on commensal microbiota, while natural Th17 (nTh17) cells acquire the capability of producing IL-17 during development in the thymus without a required differentiation step in the periphery. While iTh17 and nTh17 cells share many features, the signaling pathways essential for their development and function are not yet well understood. Using both genetic and pharmacological modulation of Akt activity, we show that Akt regulates the development of both nTh17 and iTh17 cells. Upon investigating the mechanism by which Akt controls the generation of both Th17 cell populations, we found that selective deficiency of mTORC1 activity did not affect nTh17 cells in contrast to the defective iTh17 cell generation in these mice (Rheb-deficient mice). The absence of mTORC2 activity, by deleting Rictor, an mTORC2-specific subunit, led to a severe defect in nTh17 cell development, while iTh17 cells were preserved in these mice. Mice receiving chronic rapamycin treatment, which has been demonstrated to inhibit not only mTORC1 but also mTORC2 activity, had greatly decreased nTh17 cells, while single dose of rapamycin, which interferes selectively with mTORC1 function, had no affect on these cells. In contrast, iTh17 cells were greatly diminished under both chronic and single dose of rapamycin treatment, supporting the distinct roles of mTORC1 and mTORC2 in controlling iTh17 versus nTh17 cell development. Finally, Akt isoform-specific activity also differentially contributes to nTh17 and iTh17 cell development. Selective deletion of Akt2, but not Akt1, resulted in defective iTh17 cell differentiation both *in vitro* and *in vivo* but preservation of nTh17 cells. Using mixed radiation bone marrow chimeras, we found that the aberrant iTh17 phenotype in Akt2-deficient mice is cell-intrinsic. Collectively, these data reveal novel mechanisms regulating nTh17 and iTh17 cell development and critical roles of Akt isoforms and the two distinct mTOR complexes in controlling the development of the Th17 cell subsets. Given the increasing interest in modulating Akt and mTOR pathways in various malignancies and the usage of mTOR inhibitors as immunosuppressants following tissue transplantation, our findings suggest that the effect on

this aspect of immune system development should be taken into consideration when targeting these signaling pathways.

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## Variability in Fusogenic Activity of Human Metapneumovirus Fusion Protein From Different Strains

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First identified in 2001, the paramyxovirus human metapneumovirus (HMPV) is a novel pathogen that causes viral respiratory disease in infants, the elderly, and immunocompromised patients worldwide and is the second most common cause of pediatric lower respiratory illness, following the closely related RSV. Despite its clinical significance, little is known about its entry pathway, complicating the search for antivirals. Of the three surface glycoproteins expressed on the viral membrane, the attachment protein, a small hydrophobic protein, and the fusion protein F, only the F protein is required for membrane fusion and infectivity. The F protein undergoes a dramatic conformational change in order to bring the viral and target cell membranes together. This conformation change in HMPV strain CAN97-83 has been shown to be triggered by low pH. However, low pH triggering varies between F proteins of different strains. In this study we characterized fusogenic activity of strains representative of all four clades using two independent fusion assays, syncytia formation and luciferase reporter gene assay. Our results suggest that both the overall fusion level and the amount of stimulation by low pH vary between F proteins of different strains. Comparison of F protein sequence will be used to identify potential critical residues.

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## Miro: A Driver of the Kinesin Motor

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Mitochondria are organelles central to both the life and death of a cell via their roles in ATP production and the initiation of apoptosis. The outer mitochondrial membrane protein Miro is a highly conserved calcium ($Ca^{2+}$)-binding GTPase that regulates mitochondrial transport, dynamics, and clearance. Miro attaches mitochondria to the microtubule-based motor kinesin-1 and is responsible for $Ca^{2+}$-dependent mitochondrial movement. Phosphorylation of Miro by Pink1 kinase and its subsequent Parkin-mediated degradation leads to clearance of damaged mitochondria. However, a structural basis for these activities is lacking. Here, we present a crystal structure of Miro that includes the $Ca^{2+}$-binding EF hand region and C-terminal GTPase domain. The structure reveals two previously unidentified "hidden" EF hands, each of which is paired with a canonical EF hand. Each EF hand pair is followed by a helix that structurally mimics an EF hand ligand. The GTPase domain, including a key nucleotide-sensing element, forms an extensive interface with one of the hidden EF hands, and a Pink1 phosphorylation site lies within that interface. Our results provide a structural basis for exploring $Ca^{2+}$, nucleotide, and phosphorylation-dependent regulation of mitochondrial function.

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## Supplying Balanced T Cell Signaling Using Combinatorial Antigen Recognition Promotes Selective Tumor Eradication

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Engineered T cell therapies are emerging as a promising treatment for cancer. When a T cell receives a TCR mediated activation signal from antigen presentation by an antigen-presenting cell, several additional co-stimulatory signals are necessary to avoid T cell anergy and create a robust immune response to antigen. Many monoclonal antibody based therapies targeting immune checkpoints can be successful in generating a successful anti-tumor response. However, constitutive manipulation of these co-stimulatory signals without providing antigen specificity may result in autoimmunity or an inability to eradicate tumor. The use of tumor-antigen specific Chimeric Antigen Receptors (CARs) expressed in T cells as cancer immunotherapies have shown efficacy in human clinical trials and have been extensively investigated by our group. A CAR consists of an antigen-specific single chain variable fragment (scFv) derived from fusing the heavy and light chains of an immunoglobulin domain of any monoclonal antibody, which is then fused to a CD8 transmembrane domain, which is then fused to various T cell intracellular signaling. Our group has demonstrated the requirement of providing both CD80 and CD137L by constitutive expression in order to eradicate human prostate tumors in mouse models using CAR modified T cells. We are now investigating the efficacy of using two chimeric receptors designed to target independent tumor antigens in order to provide all three activation and co-stimulation signals in an antigen-specific manner. Our data provides proof-of-principle evidence for achieving two complementary outcomes that determine specificity and safety of T-cell tumor therapy: (i) the ability to harness combinatorial antigen recognition to design T cells specific for a tumor in the absence of a truly tumor-specific target antigen and (ii) the ability to protect cells that express only one of the targeted antigens by titrating activation and costimulatory signals so as to confine T-cell activation to sites of target antigen coexpression.

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## Neddylation Inhibitor MLN4924 Induces Apoptosis in Acute Myeloid Leukemia Cells

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Addition of the Nedd8 molecule is a posttranslational modification required to activate Cullin RING Ligases (CLRs). CLRs are E3 Ubiquitin ligases responsible for targeting multiple substrates, including transcription factors, cell cycle regulators, and proteins involved in maintaining cellular homeostasis, for proteasomal degradation. Because excess degradation of several of these substrates has been shown to contribute to tumorigenesis and cancer progression, inhibiting the CLRs that contribute to their

degradation is an attractive chemotherapeutic option. MLN4924 is a novel small molecule that has been shown to inhibit Neddylation of CLRs and cause subsequent accumulation of these critical substrates. Most importantly, restoration of these substrates has previously been shown to be exert anti-tumor effects in multiple cell types via a variety of mechanisms. Toxic cellular effects have been attributed to DNA re-replication, apoptosis, and generation of reactive oxygen species. Our laboratory, which specializes in elucidating apoptotic chemotheraupeutic mechanisms in hematological malignancies, has begun investigating the specific mechanism of action by which MLN4924 induces apoptosis in acute myeloid leukemia (AML). Using several established AML cell lines, we are able to demonstrate robust induction of apoptosis via propidium iodide staining and flow cytometric cell cycle analysis within 48 hours of treatment with MLN4924. Because DNA re-replication was not observed, we focused our studies on the mechanism of action governing apoptosis. Preliminary data suggests the cytotoxic effect is exerted via modulation of Bcl-2 family member proteins. More specifically, the pro and anti-apoptotic proteins in this family are altered to create a cellular milieu that favors induction of apoptosis via the intrinsic mitochondrial pathway. Ongoing studies are attempting to i) determine whether these changes reflect direct alterations in turnover of Bcl-2 family members as opposed to gene expression and ii) extend these results to clinical AML samples exposed to MLN4924 *ex vivo*.

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## A High Throughput Screen to Identify Novel Modulators of Manganese Transport as a Neuroprotective Strategy for Parkinson's Disease

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The pathogenesis of several neurodegenerative diseases is modulated by complex interactions between genetic and environmental influences. Manganese (Mn) exposure has been implicated as an environmental risk factor for Parkinson's disease (PD), a neurodegenerative disorder associated with loss of the dopaminergic neurons of the substantia nigra pars compacta (SNpc). Exposure to high Mn levels in the environment, occupational setting, or disease state is followed by Mn accumulation in the SNpc, globus pallidus, and striatum; areas highly sensitive to oxidative injury and stress. Despite this well-established relationship, few therapeutic neuroprotective strategies to limit cellular Mn accumulation exist. The advent of high throughput screening (HTS) technology permits identification of compounds that influence various cellular phenotypes. However, screening for small molecule chemical modifiers of neurotoxicants has been limited by the scalability of existing phenotyping assays. Furthermore, the adaptation of existing cellular assays to HTS format requires substantial modification of experimental parameters and analysis methodology to meet the necessary statistical requirements. Here we describe the successful optimization of the Cellular Fura-2 Manganese Extraction Assay (CFMEA) for HTS. By optimizing cellular density, manganese (Mn) exposure conditions, and extraction parameters, the sensitivity and dynamic range of the fura-2 Mn response was enhanced to permit detection of positive and negative modulators of cellular Mn status. Finally, we quantify and report strategies to control sources of intra- and interplate variability by batch level and plate-geometric level analysis. Our goal is to enable HTS with the CFMEA to identify clinically relevant novel modulators of Mn accumulation. Furthermore, on going and future studies will utilize human induced pluripotent stem cell (hiPSC) technology to generate midbrain dopaminergic (DAergic) neuronal precursors for Mn toxicity experiments. Compounds identified from the CFMEA HTS, will be used to study cellular Mn handling in hiPSC-derived midbrain DAergic precursors. These cells will be used to characterize the profile of Mn neurotoxicity and neuronal response to mitochondrial stress. Support: NIH T32 GM07347 (KKK), NIH P30 ES000267 (ABB), NIH RO1 ES016931 (ABB), NIH R01 ES010563 (ABB/MA).

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## Examining Cortical Recruitment in Tasks Requiring Shift from Local to Global Processing in Children with Autism

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**Background:** Enhanced performance of individuals with autism on tasks such as the Embedded Figures Test (EFT) and the Block Design Test (BDT) has been attributed to their increased reliance on local details (Plaisted et al, 2003; Mottron et al, 2003). There are only a few neuroimaging studies targeting global and local processing in autism (Damarla et al., 2010; Liu et al., 2011; Ring et al., 1999), with main findings of increased posterior brain activation in participants with autism. While these studies have focused on adults with autism, the main goal of the present study is to probe the neural circuitry underlying the global and local processing in children with autism. **Objective:** The main objective of this fMRI study is to examine the neural bases of global and local processing in children with autism. Methods: Eleven high-functioning children with autism (age range: 10-15 years) and thirteen age-and-IQ-matched typical control participants took part in this fMRI study. The stimuli, presented in an event-related design, consisted of larger geometric shapes made out of different smaller geometric shapes. Participants were prompted to identify the bigger picture in some trials (global condition) or alternatively to identify the smaller components of the bigger picture in the remaining trials (local condition). The fMRI data collected from a Siemens 3.0T MRI scanner were analyzed using Statistical Parametric Mapping (SPM8). **Results:** Analysis of behavioral data revealed intact task performance in participants with autism with no significant group difference in accuracy (Control: Local-83%, Global-86%; Autism: Local-79%, Global-77%) or latency (Control: Local-2606ms, Global-2648ms; Autism: Local-2534ms, Global-2358ms). Within group brain responses suggest robust activation in superior parietal and occipital areas in both autism and control groups during local and global processing. Between group contrasts revealed significantly greater activation in autism in bilateral precuneus, right middle temporal gyrus, and right lingual gyrus during global processing ($p<0.005$, cluster size=90 $mm^3$). Further analysis using parameter estimates also showed increased recruitment of the precuneus in autism during global processing. **Conclusions:** A more expansive

pattern of brain activation in global processing in autism may imply the need for participants with autism to recruit more areas in order to overcome a potential default local-oriented processing. It should be noted that the behavioral performance was intact, but not superior, in participants with autism both in local and in global processing, perhaps attributed to a local advantage manifested only in open tasks (Plaisted, 2001). Increased activation in autism in the lingual gyrus and right middle temporal area is perhaps indicative of more effort in global processing (Fink et al., 1996; Han et al., 2002; Seymour, 2008). In addition, precuneus activation in autism suggests the shift in attention from local (default in autism) to global shape (Himmelbach et al., 2009). Therefore, the shift from local to global processing in autism may require additional effort at the neural and cognitive levels.

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## Chain Composition of Primary Dietary Fats Mediates Impaired or Preserved Triglyceride Dynamics, Contractility, and Nuclear Receptor Activation in Decompensated Hearts

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Oxidation of long-chain fatty acids (LCFA) provides approximately 70% of ATP in the heart. Alternatively, LCFAs incorporate into the endogenous lipid pool as triacylglyceride (TAG). The dynamic process of TAG turnover is reduced in cardiac hypertrophy, preventing contribution of TAG to beta oxidation. Additionally, reduced TAG turnover limits the availability of ligand for activation of the nuclear receptor peroxisome proliferator-activated receptor alpha (PPAR-alpha), reducing the expression of target genes involved in LCFA storage and metabolism. The current study tested the hypothesis that LCFA storage kinetics are influenced by acyl chain composition by comparing incorporation of two major plasma LCFAs, palmitate (16-carbon, saturated) and oleate (18-carbon, monounsaturated), into the TAG pool of sham operated control (SHAM) and hypertrophied (HYP) rat hearts. Isolated hearts were perfused with buffer containing 5 mM glucose, 1 mM lactate and 0.4 mM of either $^{13}$C-palmitate or $^{13}$C-oleate. Sequential $^{13}$C NMR of intact hearts and endpoint LC/MS enabled quantitation of TAG dynamics. With palmitate, HYP hearts contained 48% less TAG vs SHAM ($P<0.01$), while oleate maintained similar TAG in HYP and SHAM. LCFA incorporation into TAG displayed two distinct kinetic components: a saturable exponential, reflecting LCFA uptake, and a linear component, reflecting TAG turnover. Time constants of the exponential uptake phase reflected similar rates of uptake of either LCFA in SHAM and HYP. However, HYP displayed reduced TAG turnover with palmitate (HYP: 46.7 +/- 12.2 nmol/gdw/min, SHAM: 84.3 +/- 4.9; $P<0.01$), while oleate supported similarly elevated turnover in both groups (HYP: 140.4 +/- 11.2 nmol/gdw/min, SHAM: 143.9 +/- 15.6). Increased rates of TAG turnover correlated with decreased $^{13}$C fractional enrichment of acetyl CoA, consistent with increased contribution of endogenous, unenriched TAG to oxidative ATP production in SHAM and HYP hearts perfused with oleate. Work output (rate-pressure-product) was reduced similarly in HYP with either LCFA. In contrast, oleate supported greater contractility than palmitate in HYP for both +dP/dt (25%) and -dP/dt (23%) ($P<0.05$). In both SHAM and HYP hearts, perfusion with oleate supported significantly higher content of PPAR-alpha target gene mRNA compared with palmitate. These data demonstrate that FA composition influences TAG turnover and PPAR-alpha activation in both normal and hypertrophied myocardium. The findings link reduced TAG turnover rates to impaired dP/dt and nuclear signaling through PPAR-alpha. In the setting of pressure-overload hypertrophy, oleate may be a preferred energy substrate to palmitate due to its ability to improve lipid content, storage dynamics, and nuclear receptor signaling.

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## Dissecting the Requirement for Multiple Espf$_U$ Repeats in Ehec Actin Pedestal Formation in Polarized Intestinal Epithelium

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Enterohemorrhagic *E. coli* (EHEC) is a major cause of severe diarrheal illness that can lead to a life threatening complication known as Hemolytic Uremic Syndrome (HUS). Upon infection, EHEC produces striking "actin pedestals" on gut epithelia by injecting two type III effectors, Tir and EspFu (also known as TccP). Tir mediates tight bacterial attachment by binding the bacterial surface protein Intimin, and also recruits the host adaptor IRSp53/IRTKS. EspFu contains a variable number of repeats, each of which consists of a proline-rich ("P") domain that facilitates recruitment by IRSp53/IRTKS, and a helical ("H") domain that activates the actin nucleation promoter, N-WASP. A single H domain can activate N-WASP *in vitro* and when artificially clustered at the plasma membrane of HeLa cells (unpub data). However, a broad survey of pedestal-forming *E. coli* showed that EspFu virtually always contains at least three repeats. The requirement for three or more repeats of EspFu in nature, in contrast to results of *in vitro* studies may hint at alternatives to our current understanding of pedestal formation by EHEC. In this study, we attempt to address the requirement for multiple repeats of EspFu for pedestal formation in intestinal epithelial by functional mutagenesis of H and P domains. EspFu derivatives with varying functional repeats were tested for the ability to generate pedestals on HeLa cells, or non-confluent (non-polarized), confluent, and polarized HCT8 intestinal cells. An EspF$_U$ derivative containing two P and one H domains could promote robust pedestals on HeLas and non-polarized HCT8, while three repeats were needed for polarized HCT8 cells. Inactivation of any two of the three H domains had little effect, indicating that a single H domain is sufficient for N-WASP activation. Inactivation of any one of the three P domains prevented recruitment of EspF$_U$ to Tir and concomitant pedestal formation in polarized HCT8. The apparent minimal EspF$_U$ allele (three repeats) observed in nature reflects a requirement for three P domains for recruitment of EspFu to sites of attachment in polarized intestinal cells. In contrast to previous *in vitro* results, our findings may suggest additional required or inhibitory factors needed for EHEC pedestal formation in polarized epithelia.

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### Protein Tyrosine Phosphatase Shp2 and Neonatal Cardiac Innervation

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Autonomic innervation of the heart begins *in utero* and continues during the neonatal phase of life. A balance between the sympathetic and parasympathetic arms of the autonomic nervous system is required to regulate heart rate as well as the force of each contraction. Our lab studies the development of sympathetic innervation of the early postnatal heart in a conditional knockout (cKO) of *Src homology protein tyrosine phosphatase 2* (Shp2). We targeted Shp2 in post-migratory NC lineages using our novel *Peri-Cre*. This resulted in a fully penetrant mouse model of diminished sympathetic cardiac innervation and concomitant bradycardia and first degree AV block that progressively worsen. Shp2 is a ubiquitously expressed non-receptor phosphatase involved in a variety of cellular functions including survival, proliferation, and differentiation. Shp2 is thought to mediate these functions through a plethora of signaling cascades including Extracellular Regulated Kinases (ERK) 1 and 2. We hypothesize that abrogation of downstream ERK1/2 signaling in NC lineages is primarily responsible for the failed sympathetic innervation phenotype. *Shp2* cKOs are indistinguishable from control littermates at birth and exhibit no gross structural cardiac anomalies; however, preliminary *in vivo* electrocardiogram (ECG) characterization revealed sinus bradycardia and first degree AV block that progressively develop as the mutant ages. Significantly, 100% of *Shp2* cKOs die within 3 weeks after birth. *R26r* reporter cre/loxP lineage mapping and 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. These preliminary data suggest that the diminished sympathetic cardiac innervation and the resulting ECG abnormalities may be directly mediated via decreased pERK signaling in post-migratory NC lineages.

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### MCTP2 is a Novel Regulator of Cardiac Development

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Left Ventricular Outflow Tract (LVOT) Defects are a subset of congenital heart defects that include bicuspid aortic valve, aortic stenosis, coarctation of the aorta, and hypoplastic left heart syndrome. LVOT defects demonstrate high levels of heritability, with elevated relative risk of disease for siblings of affected individuals. Despite a strong indication of genetic contribution, only a few causative genes, such as *NOTCH1*, have been identified. Screening a cohort of patients with LVOT defects, our lab previously identified genetic copy number variants and point mutations in the gene encoding MCTP2 (Multiple C2 Domains Transmembrane Protein 2), a transmembrane Calcium-binding protein. Little is known about the cellular or developmental function of this gene. To identify a potential role for MCTP2 in cardiac development, we analyzed the developmental expression pattern, subcellular localization, and functional role of MCTP2. Immunohistochemistry and RT-PCR demonstrate that MCTP2 is expressed in the developing mouse heart at time points critical for left ventricular outflow tract development. Further, we demonstrate that MCTP2 co-localizes *in vitro* with RAB5, an endosomal marker, and with extra-nuclear NOTCH1. Loss of MCTP2 dysregulates NOTCH1 signaling in endothelial cells. We additionally demonstrate that loss of MCTP2 *in vivo* leads to defects in cardiac development in *Xenopus laevis* and mouse. These findings implicate *MCTP2* as a crucial gene in heart development, and suggest that MCTP2 may interact with NOTCH1 trafficking to regulate LVOT formation.

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### Vitamin D Supplementation Improves Cognitive Function and Alters Hippocampal Gene Expression in Aging Rats

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There is growing concern regarding the extent of Vitamin D (VitD) deficiency in the general population, but especially in the elderly who are at greatest risk. Despite the prevalence of VitD deficiency in the elderly, relatively little is known about how VitD affects the brain and cognitive function. Based on our prior *in vitro* and *in vivo* studies, we suspect that increased VitD levels may reverse markers of brain aging and counteract some aspects of age-related brain decline. Because this decline begins to appear around midlife, this age may represent a critical window of opportunity for the manipulation of VitD status. In order to test the hypothesis that age-related brain decline is slowed or prevented by higher VitD levels, dietary manipulation of VitD was initiated at midlife and continued for 4-5 months. Male rats (12 months old) were divided into three groups and fed diets containing varying amounts of VitD: low, normal (conventional), or high. Following chronic treatment, a memory-based water maze task was used to provide potential insight into how VitD status may affect age related brain decline. This task required the rats to find a hidden platform in a pool of water using visual cues placed around the pool. Additional microarray studies were performed to identify potential gene pathways targeted by VitD. Our studies indicate that VitD status affects cognitive function during aging. We found that maintaining higher levels of VitD during middle-age appears to be an important factor in preserving and extending healthy brain function and cognitive ability. Specifically, middle-aged rats maintained on the high VitD diet were more successful in the memory-based task than the normal and low VitD fed rats. Additionally, microarray analysis revealed selective expression of genes involved in synaptic function in the hippocampus of animals fed the high VitD diet. This alteration in hippocampal gene expression may represent an underlying mechanism by which VitD appears to improve the likelihood of successful brain aging.

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## The Role of Specific c-Jun N-terminus Kinase Isoforms in Huntington's Disease Pathogenesis

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Huntington's disease (HD) is an adult onset neurodegenerative disease caused by a mutation encoding an expansion of a polymorphic polyglutamine (polyQ) tract in the huntingtin (Htt) protein. HD is inherited in an autosomal-dominant manner with 100% penetrance. Symptoms include motor and cognitive deficits that increase in severity until death, usually around 20 years after diagnosis. At present, there are no disease-modifying treatments for HD. Despite ubiquitous expression of Htt, degeneration is mainly observed in striatal neurons. The unique morphology of neurons renders them distinctly vulnerable to deregulation of axonal transport of membrane-bound organelles (MBO) and kinase-based signaling mechanisms. PolyQ-Htt inhibits MBO axonal transport in squid axoplasm and mammalian cultures, and various reports showed activation of the c-Jun N-terminus kinase (JNK) pathway by polyQ-Htt, and a protective effect of JNK inhibition in cellular and animal HD models. Mass spectrometry studies showed that recombinant JNK3 phosphorylates Ser176 in the microtubule-binding region of kinesin-1. These data suggest that specific JNK isoforms may play a role in the axonal transport deficits observed in HD. Isoform-specific JNK shRNAs were used to knock down JNK1 or JNK3 in N2a cells expressing wt- or polyQ-Htt. Axonal transport deficits were evaluated by neurite outgrowth and neurite mitochondrial distribution. Results from both outcomes provide statistically significant data to indicate that JNK3, and not JNK1, mediates polyQ-Htt induced deficits in axonal transport. By better understanding the underlying molecular pathogenic mechanism for HD, we identify potential therapeutic targets for a devastating disease.

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## Dgcr8-ablated Schwann Cells Display Defective Myelination

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Background: Schwann cells (SCs) play important roles in the peripheral nervous system. As demonstrated in many demyelinating neuropathies, proper differentiation of SCs is essential for producing myelin sheaths around axons to increase the speed of axon conduction and allow proper function of the nervous system. Since there are many human diseases and conditions, such as congenital hypomyelination, Charcot-Marie-Tooth disease and nerve trauma, that can be ameliorated through enhancing SC functions, it is imperative to understand the molecular mechanisms underlying SC differentiation. Recently, our group and others have found that microRNAs are crucial in the regulation of SC differentiation during development (Yun et al, J Neurosci, 2010; Perreira et al, J Neurosci, 2010; Bremer et al, PLoS ONE, 2010; Verrier et al, J Neurosci res, 2010). Mice with SCs lacking *Dicer1* (*Dicer1* cKOs), which is believed to be required for processing most of the miRNAs, resemble the phenotype of Egr2-deficient mice, both of which display a severe neurological impairment mimicking congenital hypomyelination. Interestingly, although the P0::Cre strain we used is active in the SC lineage from midgestational time points (13.5 d postcoitum) and the *Dicer1* cKOs' movement is already visibly impaired between P7 and P14, we were surprised to find that only 25 out of 518 microRNAs tested in P7 *Dicer1* cKOs sciatic nerves were reduced by greater than 10 fold by Taqman array microRNA cards. One possible explanation is that DICER1 or many microRNAs exhibit very long half-life in SCs. Methods: We obtained mice with SCs lacking *Dgcr8* (*Dgcr8* cKOs) by crossing P0::Cre strain to *Dgcr8*$^{f/f}$ strain. We have characterized *Dgcr8* cKOs using behavioral tests and standard molecular biology methods, including electron microscopy, Taqman array microRNA cards, qRT-PCR, and immunohistochemistry. Results and Conclusions: *Dgcr8* cKOs exhibit impaired myelin development and display severe neurological phenotypes that are similar to *Dicer1* cKOs. Funding sources: This work was supported by National Institutes of Health Grants 1R01NS071081-01.

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## Mapping Unique Interaction Domains in Sterol Biosynthetic Complexes for the Design of Novel Anti-Fungal Reagents

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In response to the ever increasing use of immunosuppressive therapies, an unprecedented number of virulent fungal diseases have emerged and now pose a significant threat to human health. This, as well as the growing resistance to current anti-fungal treatments, highlights the need to develop novel broad-spectrum pharmaceuticals. Rather than targeting the active sites of the enzymes involved in the sterol biosynthetic pathway, we suggest a closer look at the possibility of disrupting macromolecular protein assemblies. Squalene synthase catalyzes the first committed step in sterol biosynthesis, and experiments using *Sacchromyces cerevisiae* have shown that a 26 amino acid sequence near the C-terminus of this enzyme is necessary for the auxotrophic complementation of sterol biosynthesis. Substitution of this sequence with a corresponding sequence from either the mammalian or plant squalene synthases does not allow the yeast to meet its sterol requirement even though the enzyme is still active and squalene accumulates. Conversely, overexpression of just the yeast 26 amino acid sequence in yeast, but not the animal or plant sequences, appears to be a lethal phenotype. These observations suggest that the short amino acid sequence is specific within each Kingdom of life and may play a role in allowing squalene synthase to integrate into a metabolic complex for sterol metabolism. Our objective is to demonstrate that this sequence mediates physical interactions with other proteins. This may then allow us to design small molecules to disrupt these interactions in a kingdom specific manner, thus providing evidence for the development of new, specific reagents to control the growth of fungal pathogens.

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### Mechanisms of Hydrocephalus in the Neuropathogenesis of Cryptococcal Meningitis

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HIV-related cryptococcal meningitis carries a 40% mortality globally even with optimal treatment and can be as high as 80% in resource limited areas. Infection induced hydrocephalus leads to cognitive impairment, progressive loss of vision, decreased level of consciousness and ultimately death if not treated. The only treatment option, combined amphotericin B, flucytosine, and fluconazole; has low efficacy, is poorly tolerated, and toxic. Many believe hydrocephalus ensues from arachnoid villi obstruction by cryptococcal capsule and a larger capsule creates a greater hindrance to spinal fluid flow. However, these assumptions have yet to be validated and a concrete mechanism outlining cryptococcal pathogenesis yet to be determined. Our intentions are to characterize the mechanisms causing hydrocephalus and subsequently, the development of meningitis. Previously, we inoculated mice with 3 isogenic *C. Neoformans* strains: normocapsular (H99), hypercapsular (PKR1-33), and hypocapsular (Cap 59). Interestingly, the normocapsular strain showed faster disease progression and higher mortality compared to the other strains. The normocapsular injected mice were grossly diseased 3 days post inoculation, showing behavioral signs of sickness and overt hydrocephalus. However, postmortem brain homogenates confirmed the expected capsule size difference between each strain, with cap 59 having the smallest capsule and PKR1-33 having the largest capsule. This demonstrates that hydrocephalus doesn't correlate to capsule size, that other factors are involved. These findings are significant and need to be investigated in order to improve treatment modalities. Our current endeavors include demonstrating direct evidence of villi obstruction, quantifying murine hydrocephalus, and evaluating the host inflammatory cytokine response. Specifically, we will use an optical sensor transducer to measure opening intracranial pressure through the cistern magna of infected mice. Evidence of villi obstruction will be evaluated via immunohistochemistry of brain sections. Also, several cytokines, including Th2, Th1, TNF-alpha and IL-10 are being studied. The results of this study will further clarify characteristics of cryptococcal virulence, contributing toward the development of medicine that could potentially mitigate the worldwide mortality burden. Thus, aiding the 1 million people annually infected by Cryptococcus.

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### Identification of Candidate Variants in Ciliopathies Using a Bayesian Neural Network

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Despite their success in identifying variants associated with complex disease, current SNP by SNP methods used in genome-wide association studies (GWAS) are inadequate for use with population casecontrol exome sequencing data. Exonic rare variants, variants with < 1% minor allele frequency (MAF), are particularly important in highly penetrant monogenic/oligogenic disease because they are predicted to have large deleterious effects. However, these variants challenge traditional frequentist approaches because of their sparsity and allelic and locus heterogeneity. Given the sparsity of rare variants in sequencing data, a statistical model should take advantage of genetic structure. For example, the model should accommodate situations where multiple variants throughout the population may affect genes and pathways at different nucleotide positions but still produce the same disease phenotypes. We implement a Bayesian neural network that jointly estimates the impact of variants, genes and pathways in impacting casecontrol status. Through the appropriate use of public databases as well as appropriate priors, these models help isolate candidates for further functional testing. We apply these methods to a highly penetrant oligogenic class of diseases, "ciliopathies" caused by functional defects to primary cilium. Primary cilia are near ubiquitous throughout the vertebrate body and play integral roles in chemical sensation, signal transduction and cell growth. Functional defects in the over 1000 proteins required for the generation and maintenance of cilia result in multiple system diseases that have high phenotypic and genetic heterogeneity. Using next generation sequencing, we interrogated 457 clinically diagnosed ciliopathy patients with unknown genetic causes on a gene panel of 810 suspected ciliopathy genes. We compare this population to 997 normal individuals from the Atherosclerosis Risk in Communities Study (ARIC) study and report several putative genetic variants for zebrafish functional studies.

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### Processing Submillisecond Timing Differences in a Model Electrosensory System

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Perception of sensory cues requires peripheral encoding followed by extraction of behaviorally relevant signal components by central neurons. Some sensory systems can detect temporal information with submillisecond accuracy, despite these signals occurring faster than the approximately 1 ms timescale of neuronal firing. Disruptions to sensory processing can affect isolated sensory modalities as in Auditory Processing Disorder or can manifest broadly as in Autism Spectrum disorders.

# Poster Abstracts

I investigated mechanisms for processing submillisecond timing differences by studying electrosensory processing in a time coding expert, mormyrid weakly electric fish, which can detect submillisecond differences in the duration of electric signals. First, I measured responses of peripheral receptors to stimuli of different durations. I found that each unit responded preferentially to longer stimuli, but with response thresholds that varied among units within the behaviorally relevant range of durations. This variability establishes a population code operating at near threshold intensities in which the number and identity of responding receptors represents duration. At higher stimulus intensities all units respond independent of duration, rendering the population code obsolete. Importantly, peripheral receptors respond either to the start or end of a signal. Thus, stimulus duration is also represented by a temporal code, as a difference in spike times between receptors. Next, I investigated the central mechanism for detection of submillisecond spike time differences by recording from time comparator neurons (Small Cells) in the midbrain. Recording from Small Cells is challenging because their somas are small and relatively inaccessible. I therefore designed a novel method using retrograde labeling to directly visualize and record from Small Cells *in vivo*. I showed that patterns of duration tuning vary among Small Cells due to a combination of blanking inhibition corresponding to one edge of a stimulus and variably delayed excitation corresponding to one or both edges of a stimulus. Other circuits that detect submillisecond timing differences rely either on precisely-timed inhibition or delay-line coincidence detection. I demonstrate a novel mechanism by which mormyrids combine delay-line coincidence detection with precisely-timed blanking inhibition to establish diverse patterns of duration tuning among a population of time comparators.

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## A Novel, Isoform-specific Interaction Between DIPA and p120-catenin is Implicated in N-cadherin Function *In Vivo*

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p120-catenin (p120) is a master regulator of cellular adherens junctions and is important for epithelial homeostasis, development, tumorigenesis, and metastasis. Relatively little is known about the different p120 isoforms, which are encoded by and named for alternative start sites (numbered 1-4) and spliced exons (lettered A-D). p120-3A and -1A are predominant in epithelial and mesenchymal cells, respectively, and isoform switching occurs during Epithelial-to-Mesenchymal Transition (EMT) concomitantly with the switch from E-cadherin to N-cadherin expression. During validation of a yeast two-hybrid screen using p120-1AB as bait, we identified the first known p120 isoform-specific binding partner, Delta-Interacting Protein A (DIPA). DIPA is a predominantly nuclear protein suggested to function in transcriptional repression. In this study, we map the interaction between p120 and DIPA and show that the p120 N-terminal head domain and both DIPA coiled-coil domains are required for full-strength binding. We observe that Flag-tagged DIPA co-localizes and co-immunoprecipitates reciprocally with over-expressed p120-1A, but not p120-3A, which lacks the N-terminal head domain. Using monoclonal antibodies that we made against full-length DIPA, we show that endogenous DIPA precisely co-localizes with p120-1A and N-cadherin in polarized and non-polarized Madin-Darby Canine Kidney (MDCK) cells. We report for the first time that endogenous DIPA localizes to adherens junctions in a p120-dependent manner. The p120 family members Delta-catenin, p0071, and Armadillo Repeat deleted in Velo-Cardio Facial syndrome (ARVCF) have similar N-terminal regions to p120-1A and also bind to DIPA. Furthermore, two DIPA family members, CCDC85A and CCDC85C, are able to bind p120-1A and its family members at cell-cell junctions, suggesting that this family of interactions is evolutionarily conserved. Finally, in a zebrafish model of neural tube development, both DIPA morpholino knockdown and mRNA over-expression phenocopy an N-cadherin mutant. DIPA is the first protein discovered to selectively interact with isoform p120-1A, and our data suggest that p120 or one of its family members recruits DIPA to adherens junctions for a developmentally important N-cadherin-specific function.

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## Structural Mechanism of How Cowpox Virus Hijacks the KDEL Receptor to Sabotage MHCI Antigen Presentation

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Viral immune evasion proteins serve to camouflage infected cells from the host immune system. When these proteins subvert MHCI antigen presentation, they specifically aid the virus in evading detection and clearance by cytotoxic T-lymphocytes (CTLs). Remarkably, immediate and latent MCMV and RhCMV infections of their respective hosts are not affected by the ablation of MHCI-specific immune evasion proteins, while CTL-dependent attenuated virulence was observed for the equivalent mutant cowpox virus (ΔCPXV012, ΔCPXV203) in murine infection. Our work here demonstrates that the CPXV203 protein directly binds mature MHCI heterodimers to bridge MHCI to the KDEL receptor (KDELR) rescue pathway via the viral protein's C-terminal ER-retention sequence (KTEL), thereby sequestering MHCI in the ER. Like KDELR/KDEL binding, the CPXV203/MHCI interaction was revealed to be pH-dependent, insuring high-affinity association in the Golgi for retrieval to the ER. Our initial structural studies of CPXV203/MHCI at low pH revealed that CPXV203 binds MHCI using a niche below the α2-1 helix conserved through both chaperone (tapasin) and cell surface (CD8 & NKR) host interactions. Cellular & biophysical studies of interface mutants identified the critical importance of CPXV203 recognition of the MHCI α3 domain. This interface includes CPXV203 histidines that confer pH-dependent binding. Our most recent structural study of this interaction at high pH supports our hypothesis that CPXV203/MHCI pH regulation involves only small local effects in the α3 interaction,

while this structure also suggests pH may help to coordinate MHCI and KDELR binding. These studies clarify mechanistically how cowpox immune evasion proteins coordinate to block antigen presentation by targeting distinct MHCI assembly states.

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## Directed Differentiation of Human Pluripotent Stem Cells Into Three-dimensional Gastric Tissue

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Lesions of the gastric mucosa, including peptic ulcer disease and gastric adenocarcinoma, are an enormous global health concern. The current *in vivo* and *in vitro* systems used to model gastric disease have significant limitations. Importantly, the bacterium *Helicobacter pylori*, the most prevalent and major risk factor for gastric disease, does not have the same pathological effects in mice as it does in humans. Thus, we have developed a *human* model of the gastric mucosa that will allow for unprecedented studies into human-specific mechanisms of gastric epithelial homeostasis, physiology, and disease. To accomplish this, we use a temporal series of growth factor manipulations that mimic *in vivo* stomach development to differentiate human pluripotent stem cells (hPSCs) into three-dimensional, functional gastric organoids. Following induction of definitive endoderm, foregut tube-like structures are generated by simultaneous manipulation of Wnt, FGF, and BMP signaling pathways. The foregut spheroids are then embedded in a three-dimensional growth matrix and patterned into presumptive antral epithelium with retinoic acid. Over the course of several weeks, the spheroids undergo dramatic growth and morphogenetic development into large gastric organoids that represent the human antrum. The organoids contain a tall columnar epithelium that is organized into primitive pits and glands, remarkably reminiscent of the E18.5 mouse antrum. The epithelium is uniformly Pdx1-positive and contains the normal antral cell types. These include Muc5AC-positive mucous cells that secrete mucus into the organoid lumen and subsets of endocrine cells that produce gastrin, ghrelin, and somatostatin. Further, to test the usefulness of these antral organoids as an *in vitro* model of human disease, we performed luminal microinjections of live cultures of *H. pylori*. By 24 hours post-infection, we have observed attachment and invasion of bacteria into gastric epithelial cells by confocal and electron microscopy, as well as stimulation of epithelial proliferation. In conclusion, the human gastric organoids are a novel system that can be used to interrogate mechanisms of human stomach development, homeostasis, and disease, as well as an *in vitro* platform for drug testing. Efforts to generate analogous fundic gastric organoids are ongoing.

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## CD8αα IEL TCRs Recognize Cognate Ligand in the Thymus with High Affinity

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The conventional T cell repertoire is shaped by positive and negative selection mechanisms in the thymus that ensure the generation of T cells capable of responding to foreign (but not self) antigens presented by major histocompatibility complex (MHC) molecules. Other T cell subsets including NKT cells, T regulatory cells, and TCRαβ$^+$CD8αα$^+$ intestinal intraepithelial lymphocytes (CD8αα IEL) are thought to utilize different pathways of development driven by the specificity of the thymocytes' T Cell Receptor (TCR). In order to study the developmental pathway of CD8αα IEL, we sequenced TCRs from CD8αα IEL and from conventional TCRαβ$^+$CD8αβ$^+$ IEL and used 5 of the sequences obtained from each to construct TCR transgenic mice. Our results suggest that TCR specificity drives commitment to the CD8αα IEL lineage as CD8αα IEL TCR transgenics generated predominantly CD8αα IEL-like cells. Further, in contrast to conventional T cells, developing CD8αα IEL recognize ligand in the thymus with high affinity as shown by high expression of proteins implicated in negative selection and strong TCR signaling including Bim, Nur77, PD-1, and CD5. Further work will seek to identify the MHC molecules capable of selecting cells in to the CD8αα IEL lineage as well as the key transcriptional regulators induced by selection.

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## Investigating the Tropic Effects of the *InlAB* Locus of *Listeria monocytogenes* Reveals Allele-Specific Contributions to Cardiotropism

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Cardiotropic strains of *L. monocytogenes* share highly related alleles of *inlA*, whose gene product is known to contribute to host cell invasion. *InlA* is located in an operon that includes *inlB*, another bacterial surface protein associated with host cell invasion. To investigate whether *inlA* and/or *inlB* contributed to cardiac colonization, the *inlA* and *inlB* alleles from the laboratory non-cardiotropic strain, 10403s, as well as the highly cardiotropic strain, 07PF0776, were inserted into plasmid vectors under the control of IPTG-inducible promoters and introduced into mutant strains of 10403s lacking the *inlAB* locus. Increased levels of either 10403S or 07PF0776 InlA reduced *L. monocytogenes* invasion of H9c2 heart cells. In contrast, increasing InlB expression enhanced bacterial invasion of heart cells, such that the 07PF0776 allele of *inlB* enhanced invasion of the 10403s-background strain to the level of 07PF0776, whereas the 10403s allele of *inlB* did not show similar enhancement. Infection of female Swiss Webster mice at sub-lethal doses indicated that mice infected with strains expressing InlB from 07PF0776 were more likely to exhibit bacterial colonization of the heart than those expressing InlB from 10403s. Mice infected with 10403S expressing 07PF0776 InlB also exhibited higher colony burdens in the heart, whereas

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liver and spleen colonization were similar between the strains. These results demonstrate that alternative alleles of *inlB* mediate different magnitudes of cardio-invasiveness *in vitro* and *in vivo*, and indicate that allele-specific variants of the *inlB* gene product alter tissue tropism for strains of *L. monocytogenes*.

## 128

## Macrophage-mediated LDL Oxidation Reduces Foam Cell Formation

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LDL oxidation is considered a critical step in the formation of foam cells within atherosclerotic lesions. Oxidized LDL binds with high-affinity to scavenger receptors that mediate uptake into macrophages and other vascular cells. Notably, these cells have also been shown to be capable of oxidizing native LDL, suggesting that LDL oxidation and foam cell generation may occur synchronously. In the current investigation we confirm that native LDL is oxidized during 1-2 day incubation with cultured bone marrow-derived murine macrophages, but we demonstrate that such oxidation is not associated with increased foam cell formation. Foam cell formation was not reduced by the antioxidant BHT despite significant inhibition of LDL oxidation. Surprisingly, when macrophage-oxidized LDL was transferred to fresh macrophages, much less cholesterol accumulation resulted compared to cells incubated with untreated LDL. This difference was not accounted for by changes in uptake of $^{125}$I-LDL, but instead was associated with a large decrease in selective CE uptake. Thin-layer chromatography suggested that reduced selective uptake was due to oxidative modification of CE that occurred during the macrophage-oxidation period. These results suggest that LDL oxidation that occurs in atherosclerotic lesions may reduce rather than increase foam cell generation. The authors declare that they have no conflicts of interest to disclose.

## 129

## Assembly and Analysis of Inversion Breakpoints in *Drosophila melanogaster* Balancer Chromosomes

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Multiply inverted chromosomes, known to *Drosophila* researchers colloquially as balancer chromosomes, are a long-established and important resource in the fly toolkit. For example, as a suppressor of recombination, the balancer allows mutant genes with deleterious phenotypes to be kept in stocks. Similarly rearranged and inverted chromosomes are also found in natural populations as well as in human disease, such as cancer. Here, we describe the assembly and analysis of a multiply inverted chromosome in *Drosophila melanogaster,* and we describe a workflow that may find clinical applications as whole-genome sequencing becomes prevalent in the clinical setting.

## 130

## Deuterium Exchange Identifies Dynamic Protein Folding Events During Infectious Prion Formation

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Creutzfeldt-Jakob disease, bovine spongiform encephalopathy, and chronic wasting disease are transmitted by infectious prions, which contain $PrP^{Sc}$, a protease-resistant form of the native cellular prion protein, $PrP^{C}$. Neither the precise composition of prions nor the atomic structure of $PrP^{Sc}$ is known, though the structure of $PrP^{C}$ has been determined. Non-protein cofactors have been found to be essential to the conversion of $PrP^{C}$ into $PrP^{Sc}$, but the events underlying this conformational change are not clear. When mixed with phospholipid and polyanion cofactor molecules, $PrP^{C}$ adopts an insoluble but non-infectious intermediate form, from which infectious prions can be generated by protein misfolding cyclic amplification (PMCA). Using deuterium exchange mass spectroscopy (DXMS) to assess the regions of protein exposed to solvent water molecules, we monitored structural changes occurring in the *in vitro* conversion process of $PrP^{C}$ through the insoluble intermediate form to $PrP^{Sc}$. These results suggest that incubation with cofactor molecules causes an initial conformational change, which appears to then permit subsequent folding events that generate $PrP^{Sc}$. In addition to clarifying molecular events during prion protein conversion, these findings identify potential sites for molecularly targeted therapies to interrupt fatal propagation if infectious prions.

## 131

## Brainstem-mediated Changes in Functionally Different Sympathetic Outputs, Mean Arterial Pressure and Femoral Artery Conductance in the Rat

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The sympathetic nervous system regulates blood pressure and blood flow through various vascular beds on a heartbeat-to-heartbeat basis. In large part, this is accomplished via adjusting sympathetic nerve influences on peripheral resistance. In particular, sympathetic control of the mesenteric vascular bed mediated by splanchnic nerves is important since 15-20% of total blood volume is contained in the viscera at rest. These mechanisms coupled, with sympathetic control of epinephrine release from the adrenal gland, play an important role in the sympathetic stress response (i.e."fight or flight"). In order to study these mechanisms simultaneously we instrumented an anesthetized rat with arterial and venous femoral catheters to measure arterial pressure (AP) and for drug administration, respectively. We then implanted a flow probe (Transonic Systems, Ithaca, NY) around the contralateral femoral artery and factored in real-time AP to measure femoral conductance. Next, we implanted silver wire electrodes on the splanchnic and adrenal sympathetic nerves. To test our measurements, we microinjected glutamate (30 nl,

100mM) into the rostral ventrolateral medulla (RVLM), a brainstem area known to directly control sympathetic nerve activity. RVLM activation produced an immediate and substantial increase in both sympathetic nerve activities (SNAs) (Splanchnic: +336%, Adrenal: +296%) and arterial pressure (+30 mmHg), coupled with a decrease in femoral artery conductance (-35%). The increase in splanchnic SNA likely contributed substantially to an increase in mesenteric vascular resistance and blood pressure. On the other hand, the decrease in femoral artery conductance may have been blunted by epinephrine release from the adrenal gland. At the same time, we know that epinephrine likely affected cardiac contractility and heart rate, although we saw very little change in heart rate here. Future studies are planned employing selective and non-selective beta adrenergic receptor antagonists to determine the contribution of epinephrine-mediated hindlimb skeletal muscle vasodilation in this response. We hope to apply this preparation in our laboratory's studies regarding the effect of chronic physical activity on control of blood pressure and sympathetic nerve activity. Funding: F30-HL105003 (NAM), R01-HL096787 (PJM).

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## Exacerbated Hyperglycemic and Inflammatory Responses are Associated with Kidney Damage After Orthopedic Trauma in Obese Zucker Rats

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Following blunt traumatic injury, obese patients have an increased risk of developing acute kidney injury compared to their lean counterparts, but the contributing mechanisms are poorly understood. We hypothesized that in a rat model of orthopedic trauma, both physiological responses and kidney dysfunction would be more pronounced in obese than in lean rats. Orthopedic trauma was elicited in obese (OZ) and lean (LZ) Zucker rats (12-13 weeks old, n=6-8 per group) via bilateral hindlimb soft tissue injury, followed by an injection of crushed bone components to the area. Glucose levels were recorded at baseline and for six hours following trauma, with glomerular filtration rate (GFR), urine albumin excretion, and plasma IL-6 levels measured before and 24 hours after trauma. LZ and OZ had similar basal fasting glucose levels, but the post-trauma hyperglycemic response was significantly greater in the OZ, with higher peak (275 ± 19 vs. 167 ± 13 mg/dL for LZ) and six-hour post-trauma levels (188 ± 8 vs. 100 ± 4 mg/dL for LZ). Compared to pre-trauma values, urine albumin excretion in the twenty hours following trauma was significantly greater in OZ (.63 ± .30 vs. 1.60 ± .51 mg) but not in LZ (.27 ± .05 vs. .51 ± .12 mg), while GFR decreased significantly after trauma in OZ (1.70 ± .13 to .89 ± .07 ml/min/g), with no changes seen in LZ (1.51 ± .15 to 1.40 ± .20 ml/min/g). IL-6 levels were similar at baseline for OZ and LZ (93 ± 28 vs. 97 ± 23 pg/mL), with OZ having significantly higher levels the day after trauma (2879 ± 780 vs. 965 ± 166 pg/mL for LZ). These results suggest that in response to orthopedic trauma, OZ exhibit exaggerated hyperglycemic and inflammatory responses, which may contribute to the acute kidney injury seen in these animals. Supported by NIH HL-51971, HL-89581, AHA-12SDG12050525.

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## Role of Deletion of Donor-Reactive T Cells in Maintaining Human Allograft Tolerance Achieved via BMT

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**Background:** The need for tolerance protocols in organ transplantation is underscored by the morbidity associated with chronic immunosuppressant use and the inability to prevent chronic rejection. Induction of donor chimerism is currently the most promising strategy to achieve renal allograft tolerance in humans. In an ITN-sponsored trial conducted at MGH, 7 of 10 combined kidney and bone marrow transplantation (CKBMT) recipients have tolerated their allograft for several years in the absence of any immunosuppressive medication, with development of donor-specific unresponsiveness in in-vitro assays. Donor chimerism was present for less than 3 weeks in each of these patients, and the precise mechanisms of tolerance are not known. While some lines of evidence support a suppressive mechanism, *in-vitro* studies also support a role for anergy or deletion of alloreactive T cells at later time points post-transplant. Assessing deletional tolerance has previously been impossible due to the unavailability of markers for the many thousands of T cell clones responding to HLA alloantigens. **Methods:** We have implemented a TCR deep sequencing approach to identify and track the alloreactive T cell repertoire between a given donor-recipient pair. In a commercially-available technique (ImmunoSEQ; Adaptive™), CDR3 regions are amplified with primers specific for all 45 known expressed Vβ and all 13 Jβ regions adapted for solid phase PCR, allowing high throughput sequencing of millions of T cell clones as well as detection of rare clones. We hypothesized that high throughput CDR3 sequencing of a CKBMT recipient's donor-responsive T cells in a one-way mixed lymphocyte reaction (MLR) prior to transplant would reveal a marked enrichment for donor-reactive TCR sequences, allowing identification of the thousands of TCRs that specifically recognize their organ donor's alloantigens and providing a method of tracking donor-reactive T cells *in vivo* in the post-transplant period. **Results:** In a study of one tolerant CKBMT patient, we identified thousands of CD4+ and CD8+ T cell clones pre-transplant that were significantly enriched (greater than 3 fold) upon exposure to irradiated donor PBMC. Of the 15 most frequent CD4 donor-reactive clones, 11 were undetectable in peripheral blood samples obtained 1.5 years post-transplant. Of the 10 most frequent CD8+ donor-reactive clones, 9 were undetectable at 1.5 years. **Conclusions:** Deletion of donor-reactive T cells plays a significant role in the maintenance of tolerance following CKBMT. Further studies are in progress in additional tolerant patients.

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### A Disparity Between Patient Perception and Provider Documentation of Bowel Dysfunction in Pregnancy

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**Objective:** To quantify provider documentation of bowel symptoms in pregnancy and to compare the rate of documentation with the patient's subjective evaluation of symptom severity and quality of life. **Study Design:** Provider documentation of functional bowel symptoms (FBS) and their management was determined by chart review. Symptom severity was assessed using the Rome III questionnaire for functional bowel disorders. Quality of life was assessed with the IBS Quality of Life Measure. **Results:** One hundred and four patients were enrolled in the study. Bowel function was documented in 64% of prenatal charts, with 94% of these documentations occurring at the intake visit. While 75% of women subjectively reported bowel dysfunction in the first trimester of pregnancy, dysfunction was only documented in only 17% of charts. There was no significant difference between either the average severity of symptoms or the quality of life of patients who reported FBS but for whom symptoms were and were not documented. Treatment of symptoms was documented only 55% of the time that FBS were recorded, with 50% of practitioners recommending medical therapy as their first line treatment. Follow up of FBS was documented in only 27% of charts in which bowel dysfunction was initially recorded. **Conclusion:** There is a large disparity in the number of women reporting FBS and provider documentation of dysfunction in the medical record. However, this disparity cannot be explained by differences in either the severity of FBS or the quality of life of those women for whom symptoms were and were not documented. It is possible that women and their providers may view FBS as a normal part of pregnancy, prompting patients to under-report FBS. The ease with which a normal review of systems can be documented in the EMR could also contribute to the under reporting of FBS. Treatment was initiated in only 50% of cases in which FBS were documented, lending further credence to the idea that medical providers may not view bowel dysfunction within pregnancy as an abnormal or high priority problem.

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### Characterization of 70k-Fibronectin interactome by IFAST Affinity Purification Coupled with Mass Spectrometry

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Fibronectin (Fn) is a large glycoprotein present in serum and extracellular matrix, important for many pathophysiologic processes. Fn contains binding domains for fibrin, collagen and heparan sulfate, and a RGD site to interact with integrins Within Fn the 70kDa N-terminus (70k-Fn) is involved in cell-mediated Fn assembly, a process required for embryogenesis, development, and platelet thrombus formation. In addition, major human pathogens including *staphlycoccus aureus* and *streptococcus pyogenes*, interact with 70k-Fn. Furthermore, *in vitro* experiments have established the importance of this interaction for the bacterial spread to distant organs. Knowledge of blood plasma and platelet proteins that interact with 70k-Fn is incomplete. In the current study, we aimed to characterize the blood plasma and platelet proteins that interact with 70k-Fn through affinity purification coupled with mass spectrometry (AP/MS). For this affinity purification, we used a novel purification technique termed immiscible filtration assisted by surface tension (IFAST). The foundation of this technology is immiscible phase filtration, using a magnet to draw paramagnetic particle (PMP)-bound analyte through an immiscible barrier (oil or organic solvent) that separates an aqueous sample from an aqueous eluting buffer. Significant energy is required to cross the immiscible phase boundary; hence this boundary functions to remove unbound proteins. To identify non-specific interactions with 70k-Fn-PMPs, we include BSA bound to PMPs as control. Using this technique, we identified 31 unique interactors from plasma serum, of which seven were previously known to interact with 70k-Fn. Similarly, four proteins were identified to interact with 70k-Fn, from platelet lysate. This is the first reported use of IFAST for AP-MS proteomic study. IFAST requires small sample volumes and is amenable to high-throughput proteomic studies. Furthermore, the use of PMPs with specific bait proteins enables quantification of interacting proteins for a specific amount of bait protein. Finally, the design of IFAST allows analysis of the remaining sample after purification, and could be extended to affinity purifications using two or more bait proteins.

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### Metagenomic Analysis of Gut Microbiome Functional Assembly in 14 Infants

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The human gastrointestinal tract is filled with trillions of microorganisms that endow metabolic and physiologic properties that are not encoded in our somatic genome. Studies in western adults have demonstrated a high level of stability of a person's microbiota over time, but little is known about the assembly of the gut microbiome in early life as the sterile fetal gut is colonized. Is there a functional core of genes found in infant microbiomes, and if so, what are the predicted properties of this community? Are there environmental factors, such as diet or malnutrition, which cause reproducible changes in microbial communities across many children? To answer these questions, we used metagenomic sequencing to characterize the early functional assembly of the gut microbiome in a birth cohort of 14 children born in Bangladesh, some of whom were healthy and some who were malnourished. Shotgun gene sequencing of the fecal DNA from stools collected at monthly intervals over the first two years of life

yielded 20.8 million high-quality reads from 242 samples (mean 86,029 ± 40,359 reads/sample; mean 360 ± 52 nucleotides/read). The reads were annotated with the KEGG and COG functional databases, along with reference microbial genomes for taxonomy assignment. Consistent with a previous time series study of a single healthy USA infant, microbiomes from younger infants were significantly enriched in genes encoding proteins involved in fatty acid metabolism and ABC transporters. Older microbiomes were enriched in a variety of metabolic pathways, notably for amino acids and carbohydrate utilization. Using a Poisson model of differential abundance, the most significantly enriched gene was sialate-o-acetylesterase (EC 3.1.1.53), which was never found before the 4th month but was detected in all samples by 2 years of age. This raises the intriguing possibility that structural variation in the host mucosa is one of the non-diet based factors driving the changes seen in the microbiota as infants age.

## 137

## Pathogenesis of Novel *FMR1* Mutations in Fragile X Syndrome

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Fragile X Syndrome (FXS) is the most common cause of inherited intellectual disability and a leading known causes of autism. Most cases of FXS are caused by trinucleotide repeat expansion within the 5'UTR of the gene *FMR1*. To date, only one missense mutation has ever been reported in a patient (I304N-FMRP). Recently we identified 2 novel variants, G266E and R138Q, in patients with FXS-like symptoms who tested negative for repeat expansion. To determine if these variants are pathological, we used lentivirus to infect *Fmr1* KO cells with G266E-FMRP or R138Q-FMRP. Interestingly, we found that G266E behaves like a functional null, similar to I304N, as it is unable to associate with polysomes or rescue AMPAR trafficking (two well-known functions of FMRP associated with regulating protein synthesis). Conversely, R138Q rescued all tested phenotypes except for synaptic overgrowth at the *Drosophila* neuromuscular junction (NMJ), indicating this variant does not impair global FMRP function but rather impairs presynaptic function specifically. These results provide information about how the different domains and structure of FMRP may be involved in FMRP's normal functions, and also give important insights into the mechanisms of disease in these patients with non-traditional *FMR1* mutations.

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## Tapping Advances in Clinical Health IT for Translational Research: Analysis of the NIH Investment in Disease Registries and the Need for Investment in Next-generation, Reusable, Multi-purpose Registry Platforms

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**Background:** The U.S. clinical health information technology landscape is now undergoing a forced modernization catalyzed by the HITECH Act of 2009, with a goal of establishing a framework capable of acquiring rich clinical phenotypes. Historically, such efforts follow a "single-purpose" model in which collection and usage of data is restricted to pre-defined protocols and recipients. We undertook a systematic, cross-sectional review of active observational registry projects funded in the U.S. to assess this landscape. **Methods:** We identified a comprehensive list of actively U.S. funded research registry-related projects for FY2009, employing structured searches of data sources at NIH and other U.S. government websites (NIH RePORTER; Catalog of NIH Funded Databases, Disease Registries, and Biomedical Information Sources). Registry projects were classified and linked to fiscal expenditures (NIH from FY1992; CDC from FY1995) using publicly available data (http://USAspending.gov; https://fpds.gov; http://tags.hhs.gov). **Results:** We identified 161 'parent' projects active in FY2009, encompassing approximately 3300 individual grants and contracts from 1992-2009. During this 18-year period, total registry project-related expenditures were $3.16B (all figures consumer price index adjusted to U.S. 2008 dollars); FY2009-only funding was $374M. Overall duration of projects was 0 to 43 (mean 6.9) years. 42% of projects were registries only; 55% included a biorepository component. NIH institute-based, central data and biorepository projects received $256M (8% total). Project funding by institute or center (IC) ranged from $801M (NCI) to <$1M (NLM, Fogarty International Center), and relatively from 2.4% (NIA) to ≤0.01% (NEI, Fogarty) as percent of total IC funding. By NIH research, condition, and disease categorization (RCDC), spending on cancer projects was $1.52B and the greatest percentage-wise registry spending represented 19% of total funding for the American Indians/Alaska Natives RCDC. A limited analysis of publication output yielded a range of 0 to 18 publications per million dollars funded for the subset of NIH grants of >2 years duration. Discussion: Registries and biorepositories represent a significant investment. Given their growing importance as sources of high-quality phenotypic data, we propose the introduction of standardized metrics for evaluating registries, common information technology platforms for interoperable data collection, and policies facilitating broad reuse of meticulously collected data.

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## Serotonin 5-HT2A Receptor Activation Potently Inhibits TNF-α Mediated Inflammation *in vivo*, and Blocks the Development of Asthma

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Inflammatory disease affects millions of people worldwide and has a great healthcare cost to both individuals and society. We have discovered that activation of serotonin 5-$HT_{2A}$ receptors has potent anti-inflammatory effects in whole animal models of inflammation. Systemic administration of the 5-$HT_2$ receptor selective agonist, (*R*)-DOI, through activation of 5-$HT_{2A}$ receptors, potently inhibits the systemic effects of TNF-α mediated inflammation and decreases expression of key proinflammatory markers like ICAM1, VCAM1, and MCP1. Furthermore, we show 5-$HT_{2A}$ activation decreases TNF-α –induced production of the circulating inflammatory cytokine IL6. These effects are potent

in vascular tissues, including the aortic arch, and superpotent in the small intestine. In the intestine, (*R*)-DOI doses as low as 0.01 mg/kg completely block the proinflammatory effects of systemic administration of TNF-α. Importantly, pre-administration of the 5-$HT_{2A}$ selective antagonist, M100907, blocks the anti-inflammatory effects of (*R*)-DOI. To further explore clinical and translational applications, we have examined the effects of 5-$HT_{2A}$ receptor activation on a common human inflammatory disease, allergic asthma. We found that in a mouse model of allergic asthma, 5-$HT_{2A}$ receptor activation with inhaled (R)-DOI completely and potently blocked the development of airways hyper-responsiveness, eosinophilia, cellular inflammation, and mucus overproduction. Together, our results have defined an exciting new role for the 5-$HT_{2A}$ receptor in inflammatory processes and indicate that 5-$HT_{2A}$ receptor activation represents a novel therapeutic strategy for treating chronic human inflammatory diseases.

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## The Role of the TET (Ten Eleven Translocation) Proteins in Human Uterine Leiomyoma

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Uterine leiomyomas or fibroids are benign smooth muscle tumors of myometrial origin; despite their benign nature, they are able to undergo rapid and significant growth. They are the most common gynecological tumors in women of reproductive age, and they become symptomatic in 25–30% of all women and in up to 70% of African American women of reproductive age. The clinical symptoms associated with uterine leiomyoma are abnormal uterine bleeding, which can lead to anemia, pelvic pressure and pain; reduced fertility; and frequent pregnancy loss. Epigenetic dysregulation of individual genes has been demonstrated in leiomyoma cells; however, the *in vivo* genome-wide distribution of such epigenetic abnormalities remains not fully understood. We have demonstrated differences in DNA methylation in leiomyoma versus normal myometrial tissues; we observed that hypermethylation of tumor suppressor genes is a common event in leiomyoma tissues compared to normal myometrial tissues. Now, we are investigating the role of the newly identified epigenetic mark, 5-hydroxymethylcytosine (5-hmc) in human uterine leiomyoma, as well as the role of the recently discovered family of Fe (II)- and α-ketoglutarate (α-KG)-dependent dioxygenases, the TET (the ten-eleven translocation) proteins (TET1, TET2 and TET3). These enzymes are able to catalyze a 3-sequential oxidation reactions: converting 5-methylcytosine (5-mC) first to 5-hydroxymethylcytosine (5-hmC), and finally 5-carboxylcytosine (5caC). We have observed a decreased in TET1 and TET3 mRNA and protein levels in leiomyoma tissues compared to normal myometrial tissues while there is no change in TET2 mRNA and protein levels. When we knocked them down (SiTET1-3) in primary human leiomyoma cells, we observed changes in PCNA and cleaved PARP. To determine a more mechanistic function, I will perform chromatin DNA immunoprecipitation (Chip) to look differential recruitment at the promoter regions of genes that I previously identified to be hypermethylated in uterine leiomyoma compared to normal myometrial tissues. Understanding the molecular mechanisms underlying the pathogenesis of uterine leiomyoma will facilitate the discovery and development of new approaches for the treatment of this disease.

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## Voxelwise Multivariate Analysis of Multimodality Magnetic Resonance Imaging

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**Objective:** Simulation studies and a data analysis are performed to explore the relative merits of various methods of controlling for the number of tests per voxel in imaging studies with multiple measurements per voxel. **Background:** Most brain magnetic resonance imaging (MRI) studies concentrate on a single MRI contrast or modality, frequently structural MRI. By performing an integrated analysis of several modalities, such as structural, perfusion-weighted, and diffusion-weighted MRI, new insights may be attained to better understand the underlying processes of brain diseases. **Design/Methods:** We compare two voxelwise approaches: (1) fitting multiple univariate models, one for each outcome and then adjusting for multiple comparisons among the outcomes and (2) fitting a multivariate model. In both cases, adjustment for multiple comparisons is performed over all voxels jointly to account for the search over the brain. The multivariate model is able to account for the multiple comparisons over outcomes without assuming independence because the covariance structure between modalities is estimated. To illustrate the power of each approach, we simulate data with multiple outcomes under four different covariance assumptions and analyze a case control study of Alzheimer's disease, in which data from three MRI modalities are available. **Results:** Simulations show that the multivariate approach is more powerful when the outcomes are correlated and, even when the outcomes are independent, the multivariate approach is just as powerful or more powerful when at least two outcomes are dependent on predictors in the model. However, multiple univariate regressions with Bonferroni correction remains a desirable alternative in some circumstances. **Conclusions:** When choosing a method to account for multiple comparisons, researchers must consider several factors, including the primary hypothesis, whether the correlations between modalities are of interest, and computational resources.

# Poster Abstracts

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## The Numerical and Functional Stability of a CD4+ "Memory" T cell Population Depends on Localized Antigen Presentation

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CD4+ memory T cells generated during acute infection slowly decline after infection is cleared. However, this issue has not been addressed for persistent bacterial infections where CD4+ T cells are the protective cell type. It was therefore of interest to assess the stability of CD4+ T cells during a persistent *Salmonella enterica* infection that is controlled by this population. We found that the population of CD4+ T cells specific for *Salmonella* peptide:MHCII ligands consisted of Th1 cells, which were numerically stable for over a year after oral infection. This stability depended on low-level persistent infection of mesenteric lymph nodes and intense proliferation by a small number of T cells in this location. Additionally, the capacity of *Salmonella* peptide:MHCII-specific T cells for IFN-γ and TNF production was maximal during low-level persistent infection but exhausted in hosts expressing systemic antigen. Thus, the stable CD4+ "memory" T cell population required for control of a phagosomal infection depended on local peptide:MHCII recognition.

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## Cellular Barcoding of Mouse and Human Mammary Epithelial Cells Reveals Large Diversity in Their *in vivo* Regenerative Activity

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**Background:** The mammary gland is composed of two lineages, basal/myoepithelial and luminal epithelial cells that together form a bi-layered branching ductal structure. There is strong evidence that these two phenotypes are hierarchically generated from a common "mammary stem cell" that has extensive self-renewal ability and a basal phenotype. However, recent evidence also suggests that the restriction of self-renewal and lineage fate in primitive mammary cells may be controlled by largely independent mechanisms resulting in subsets of cells with variable histories. **Methods/Results:** To obtain more detailed information about these mammary cell fate decisions and the extent of their variability *in vivo*, we have developed and used a clonal tracking strategy and data analysis methodology based on the lentiviral-mediated insertion of a 27 base pair non-coding DNA barcode into large numbers of test cells whose clonal progeny can then be monitored after varying periods of time post-transplant (in syngeneic or immunodeficient mice). From an experiment in which we transduced mouse basal mammary epithelial cells and then transplanted them into 2 mice, we identified 86 clones. These clones were highly diverse in size and content and included most possible distinct patterns of lineage differentiation. For example, although many regenerated subclones in secondary mice and sustained their lineage differentiation behavior during this process, others did not (e.g. clones with predominantly luminal progenitors and mature cells in a primary transplant but many basal and luminal cells in a secondary transplant), indicating that a clone consisting predominantly of differentiated cells can maintain initially undetectable cells with self-renewal activity. Analysis of 57 clones similarly derived from normal human basal mammary epithelial cells transplanted under the renal capsule of immunodeficient mice revealed human mammary cells with robust primary and secondary mammary regenerative activity, in spite of a continuing poor output of more differentiated cells. **Conclusions:** Our findings reveal an unexpected diversity of clonal behaviour of primary normal mammary epithelial cells with significant implications for understanding the regulation of normal and malignant populations with proliferative ability.

## 145

## BODIPY-xyloside Reveals Increased GAG Production in Response to Mechanical Damage in the Vestibular Labyrinth

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**Introduction:** Disrupted GAG metabolism is associated with decreased or loss of hearing and balance. Although the importance of specific GAGs and GAG metabolism has been studied previously, a method of observing real-time GAG turnover has not yet been described. We have recently synthesized a novel compound consisting of a BODIPY molecule conjugated to xyloside. The xyloside component primes the production of GAGs including heparan sulfate, dermatan sulfate, and chondroitin sulfate, while the BODIPY conjugation allows for subsequent GAG visualization under fluorescence microscopy. We hypothesized that VY-IIB 57A will prime and specifically label GAGs formed after its administration into endolymph. Here we report control expression patterns and preliminary results showing upregulation of GAG expression following mechanical insult. **Results and Conclusion:** VY-IIB 57A administered into endolymph primes GAGs and produces fluorescent GAG expression. Contrasting VY-IIB 57A signal levels represents differential GAG density and suggests that the ongoing process of GAG deposition may occur at differing rates between vestibular structures. For example, the crista's high signal suggests either a high rate of GAG deposition or greater GAG expression per unit volume within the hair cells compared to the cupula. Mechanical damage to the ampulla increases GAG expression and causes fluorescent puncta to form in the cupular area, suggesting an upregulated repair process that works to restore the cupula's structural integrity. Future studies will explore this process and the cells responsible.

# Poster Abstracts

## 146

### Downregulation of JCV T-antigen by Hypoxia and Glucose Deprivation in Medulloblastoma

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Recent studies have reported the detection of the human neurotropic virus, JCV, in a significant population of brain tumors, including medulloblastomas. Expression of the JCV early protein, T-antigen, which has transforming activity in cell culture and in transgenic mice, results in the development of a broad range of tumors of neural crest and glial origin. Evidently, the association of T-antigen with a range of tumor-suppressor proteins, including p53 and pRb, and signaling molecules, such as β-catenin and IRS-1, plays a role in the oncogenic function of JCV T-antigen. We demonstrate that T-antigen expression is suppressed by hypoxia and glucose deprivation in medulloblastoma cells and in glioblastoma xenografts that both endogenously express T-antigen. Mechanistic studies indicate that hypoxia-mediated T-antigen downregulation is due to ubiquitin-mediated degradation and that glucose deprivation-mediated suppression of T-antigen is partly influenced by 5'-activated AMP kinase (AMPK), an important sensor of the AMP/ATP ratio in cells. In addition, glucose deprivation-induced cell cycle arrest in the G1 phase is blocked with AMPK inhibition, which also prevents T-antigen downregulation. Furthermore, T-antigen prevents G1 arrest and sustains cells in the G2 phase during glucose deprivation. On a functional level, T-antigen downregulation is partially dependent on reactive oxygen species (ROS) production glucose deprivation, and T-antigen prevents ROS induction, loss of ATP production, and cytotoxicity induced by glucose deprivation. We have also found that T-antigen is downregulated by the glycolytic inhibitor, 2-deoxy-D-glucose (2-DG), and the pentose phosphate inhibitors, 6-aminonicotinamide and oxythiamine, and that T-antigen modulates expression of the glycolytic enzyme, hexokinase 2 (HK2), and the pentose phosphate enzyme, transaldolase-1 (TALDO1), indicating a potential link between T-antigen and metabolic regulation. These studies point to the possible involvement of JCV T-antigen in medulloblastoma proliferation and the metabolic phenotype and may enhance our understanding of the role of viral proteins in glycolytic tumor metabolism, thus providing useful targets for the treatment of virus-induced tumors.

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### Effect of Chronic High-dose Vicodin on Pain Sensitivity in Rats

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Vicodin, the combination of acetaminophen and the opioid hydrocodone, is one of the most prescribed drugs on the market today. Chronic use of opioids has been shown to produce a phenomena known as Opioid Induced Hyperalgesia (OIH). OIH is a condition in which opioid users experience an increased sensitivity to pain while on an opioid regiment and/or after withdrawal. While selected opioids have been shown to produce OIH symptoms in an animal model, hydrocodone and the combination drug Vicodin have yet to be studied. In this study, animals were randomly assigned to one of four groups-vehicle control, Vicodin (acetaminophen/hydrocodone), acetaminophen, or hydrocodone. The drugs were administered daily for 120 days via oral gavage. After exposure to the drugs for 120 days, the rats were tested for tactile and thermal responsiveness. The results showed that the Vicodin group of rats presented with hypersensitivity to thermal pain while on the drug. The rats receiving acetaminophen, hydrocodone, and vehicle control showed no significant hypersensitivity to thermal pain. Additionally, the Vicodin group displayed behavioral signs of OIH as they were sensitive to touch while being held. The growing use of Vicodin to treat chronic pain necessitates further research exploring the onset of this pain hypersensitivity. Defining this relationship in humans may help reduce the vicious cycle between pain hypersensitivity and Vicodin abuse.

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### Paracrine Wnt/β-Catenin Signaling Between Stem Cells and Main Population in Uterine Fibroid Cell Growth

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**Background:** Each uterine leiomyoma is thought to be a benign monoclonal tumor arising from a single transformed myometrial smooth muscle cell, however, it is not known what leiomyoma cell type is responsible for tumor growth. Recently, we have reported that leiomyoma side population cells (LMSP), which have tumor initiating cell characteristics, are necessary for *in vivo* growth of leiomyoma xenograft tumors. Lower estrogen (E) and progesterone (P) receptor (R) levels in LMSP suggest an indirect paracrine effect of steroid hormones on stem cells via the mature neighboring cells. **Results:** ERα and PR levels were strikingly lower ($p<0.05$) in LMSP compared with main population cells of leiomyoma tissues. Xenografts under the kidney capsule of immunodeficient mice made of freshly-isolated leiomyoma side population and myometrial smooth muscle cells grew to relatively large tumors (1.5 $mm^3$), whereas leiomyoma main population/myometrial smooth muscle cell xenografts produced smaller tumors (0.3 $mm^3$, $p<0.05$, $n=8$), in the presence of E+P. LMSP by itself did not grow on cell culture plates. Intriguingly, E+P induced minimal growth of leiomyoma side population when co-cultured with myometrial cells maintained in a separate insert. However, LMSP showed a robust growth when in direct contact with myometrial cells in mixed cultures; E+P further stimulated this growth. Co-culture experiments in the presence of E+P further revealed that myometrial cell-derived paracrine factors stimulate the canonical Wnt pathway in LMSP leading to transcriptional activation of Wnt/b-catenin target genes. Interestingly, it has been reported that dysregulated Wnt signaling in the uteri causes mesenchymal tumorigenesis. Activation of β-catenin via E+P promoted LMSP proliferation. Separately, a Wnt ligand array was performed on cultured myometrial cells treated with or without E+P. Validation of select genes from the PCR profiling data demonstrated significantly enhanced expression of Wnt11 and Wnt16 ($p<0.05$) in myometrial cells treated with E+P. **Conclusion**: Our findings are suggestive of a paracrine role

of Wnt ligand, which arises in response to E+P, from myometrial smooth muscle cells and activates b-catenin via enhancing its nuclear translocation in leiomyoma stem-like tumor initiating cells, eventually leading to tumor growth. NICHD 5P01HD057877.

## 149

## Development of a Highly Protective Combination Monoclonal Antibody Therapy Against Chikungunya Virus

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Chikungunya virus (CHKV) is a mosquito-transmitted alphavirus that causes global epidemics of a debilitating, often chronic polyarthritis in humans. Over five million people in Africa and Asia have been infected since 2005 so there is a pressing need for the development of therapeutic agents. We identified 230 new anti-CHKV monoclonal antibodies (MAbs) and tested their ability to inhibit infection of all three CHKV genotypes (East/Central/South African, West African and Asian). We discovered that 36 of these MAbs inhibit Chikungunya infection and almost half of them have EC50 values of less than 15 ng/mL. Four of these neutralizing MAbs provided complete protection as prophylaxis in highly susceptible immunocompromised mice and mapped to distinct epitopes on the E1 and E2 structural proteins. We identified escape mutants in brains and muscle of the few dying mice, and also generated escape mutants *in vitro*. The most protective MAb was humanized, shown to block viral fusion, and require Fc effector function for optimal activity *in vivo*. In post-exposure therapeutic trials, administration of a single dose of a combination of two neutralizing MAbs targeting different domains of the E2 surface glycoprotein or targeting both the E1 and E2 glycoproteins limited the development of resistance and protected immunocompromised mice against disease when given even 24 to 36 hours before CHKV-induced death. Selected pairs of highly neutralizing MAbs may be a promising treatment option for CHKV in humans.

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## Development of Genetically Engineered T Cell Receptor-Transduced T cells for Immunotherapy of Chronic HBV and HCV Infections

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Chronic HBV and HCV infections are significant health problems worldwide with more than 350 million people chronically infected. Although there is a preventive vaccine for HBV, there is no way to cure patients chronically infected with HBV. Furthermore, there is no vaccine for HCV, which establishes chronic infection in a high percentage of patients. Forty percent of chronically infected patients do not respond to PEG-IFN and ribavirin treatment. Spontaneous clearance of chronic HBV and HCV does rarely occur and requires a vigorous CD8 T cell response. Therefore, we use adoptive T cell therapy with TCR-engineered lymphocytes to bolster the low numbers and impaired functions of virus-specific T cells in chronically infected patients to restore the immune response. Specifically, we identified and isolated MHC class I-restricted HBV- and HCV-specific T cell receptors (TCRs) with high avidity and anti-viral activity from patients and chimpanzees who spontaneously cleared infections. These TCRs were cloned into retroviral vectors and reexpressed in chimpanzee blood lymphocytes. The transduced T cells gained a broad spectrum of effector functions that were specific to the cognate antigens of the re-expressed HBV and HCV-specific TCRs and expanded to high numbers. Collectively, our results demonstrate that the combination of retroviral TCR gene transfer together with IL-21/IL-15 stimulation can efficiently redirect the antigen specificity of resting primary T cells and generate a large number of functional effector T cells. These expanded effector T cells will be used to restore the virus specific immune responses of chimpanzees with chronic HBV- and HCV infections.

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## Ire1$\alpha$, ± Induces Thioredoxin-interacting Protein to Activate the NLRP3 Inflammasome and Promote Programmed Cell Death During Endoplasmic Reticulum Stress

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When unfolded proteins accumulate to irremediably high levels within the endoplasmic reticulum (ER), intracellular signaling pathways called the unfolded protein response (UPR) become hyperactivated to cause programmed cell death. We discovered that thioredoxin-interacting protein (TXNIP) is a critical node in this "Terminal UPR." TXNIP becomes rapidly induced by IRE1$\alpha$, an ER bifunctional kinase/endoribonuclease (RNase). Hyperactivated IRE1$\alpha$ increases TXNIP mRNA stability by reducing levels of a TXNIP destabilizing micro-RNA, miR-17. In turn, elevated TXNIP protein activates the NLRP3 inflammasome, causing Caspase-1 cleavage and interleukin 1$\beta$ (IL-1$\beta$) secretion. *Txnip* gene deletion reduces pancreatic $\beta$-cell death during ER stress, and suppresses diabetes caused by proinsulin misfolding in the Akita mouse. Finally, small molecule IRE1$\alpha$ RNase inhibitors suppress TXNIP production to block IL-1$\beta$ secretion. In summary, the IRE1$\alpha$-TXNIP pathway is used in the terminal UPR to promote sterile inflammation and programmed cell death, and may be targeted to develop effective treatments for cell degenerative diseases.

# Poster Abstracts

## 152

### Prediction of Tumor Associated Macrophage Proteolytic and Metastatic Potential Using Multivariate Kinases Analysis

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Patient-to-patient variability in disease progression continues to complicate clinical decisions in diagnosis and treatment for various cancers. An individual's biochemical milieu of cytokines, growth factors, and other stimuli contain a bevy of cues that pre-condition cells and induce patient variability in response to disease progression or treatment. We focused on monocyte-derived macrophages' (MDMs') response to these cues and their production of cysteine cathepsins, powerful proteases identified as most potent mammalian collagenases and elastases, as contributing sources to variability, as MDMs enter tumors, and assist tumor invasion and metastasis by locally degrading extracellular matrix. We have used multivariate analysis of the dynamic kinase signaling network in differentiating monocytes to predict patient-specific cathepsin proteolytic activity. Here, we hypothesize that this multivariate kinase analysis of differentiating monocytes can interpret inherent variability in patient-specific cues to predict tumor metastatic potential. To test this, primary MDMs were co-cultured with MCF-7 breast cancer cells, and invasion through extracellular matrix was quantified. MDMs increased MCF-7-cell invasion, and across the patients in this study, cathepsin activity varied by as much as 7-fold difference. We are currently correlating patient cathepsin activity levels with MDM-assisted tumor invasion. Our results suggest that patient-specific kinase analysis of MDMs could be useful in clinical settings to predict tumor associated macrophage-assisted metastasis and may inform personalized courses of more aggressive treatment for patients with potentially more invasive tumors.

## 153

### A Novel Interaction Between the Desmosomal Protein, Desmoplakin, and EB1

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Desmoplakin (DP) is an obligate component of desmosomes, intercellular junctions that are critical to the mechanical integrity of tissues such as the skin and heart. DP and other desmosomal proteins are commonly mutated in arrhythmogenic right ventricular cardiomyopathy (ARVC), a cardiac disease associated with sudden death. DP is also targeted in numerous cancers. Cancer and ARVC commonly involve misregulation of connexins, which comprise gap junctions to provide open electrical communication between neighboring cells. A yeast-two-hybrid screen was conducted to identify binding partners of DP that could elucidate its importance in gap junction regulation and disease development. Among the partners identified was the microtubule plus-end binding protein EB1 (end-binding 1), which has been shown to promote border localization of the gap junction protein connexin 43 (Cx43). Interference with EB1 binding and localization could thus be a potential mechanism by loss or mutation of DP contributes to ARVC and cancer pathogenesis. The DP-EB1 interaction was confirmed by co-immunoprecipitation of endogenous DP and EB1. His-tagged EB1 constructs were used to demonstrate that the DP N-terminus binds full-length EB1 but not the EB1 head domain, suggesting that DP interacts with the EB1 C-terminus. In situ interaction of DP and EB1 was verified using a proximity ligation assay, in which DNA oligonucleotides produce a fluorescently detectable signal if two antigens of interest are in close proximity. Structured illumination and confocal microscopy were used to test if DP governs EB1 localization. Whereas control cells demonstrated a perpendicular alignment of EB1 comets with respect to cell-cell contacts, DP-deficient cells demonstrated a parallel alignment of EB1 comets and a loss of microtubule (MT) association with junctions. Gap junction assembly utilizes MT-based trafficking to the cell membrane; accordingly, cultured cardiac myocytes demonstrated a significant reduction in Cx43 border localization upon DP knockdown. Organotypic raft cultures, in which primary keratinocytes are lifted to an air-liquid interface to differentiate into a model of stratified epidermis, were used to confirm that DP knockdown led to a significant reduction of Cx43 border localization. Collectively, these results suggest potential mechanisms by which DP regulates MT organization and gap junction assembly, and by which loss or mutation of DP contributes to disease development.

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### KSHV LANA-1 Interacts with Multifunctional Angiogenin to Utilize its p53 Interaction and Anti-apoptotic Functions

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KSHV infection and the expression of LANA-1 up-regulates the multifunctional 14-kDa angiogenin (ANG) which is detected in KS lesions and in KSHV+ primary effusion lymphoma (PEL) cells. ANG knockdown or inhibition of ANG's nuclear translocation resulted in decreased LANA-1 gene expression and reduced KSHV infected endothelial and PEL cell survival (Sadagopan et al., J. Virology. 2009 and 2011). Studies here demonstrate that LANA-1 and ANG colocalize and co-IP in de novo infected endothelial cells and in latently infected TIVE-LTC and PEL (BCBL-1 and BC-3) cells. LANA-1 and ANG interaction occurred in the absence of KSHV genome and other viral proteins. ANG co-eluted with LANA-1, p53 and Mdm2 in high molecular weight fractions. LANA-1, p53 and Mdm2 also co-IPed with ANG and LANA-1, ANG and p53 colocalized in KSHV + cells. Colocalization between ANG and p53 was also observed in KSHV negative cells. Silencing endogenous ANG in KSHV negative cells induced p53 promoter activation and p53 target gene (p53, p21 and Bax) expression, downregulated anti-apoptotic Bcl-2 gene expression and increased p53-mediated cell death. In contrast, ANG expression blocked Bax and p21 expression, induced Bcl-2 and blocked cell death. ANG expression in KSHV negative cells also resulted in the inhibition

of p53 phosphorylation, increased p53-Mdm2 interaction, and increased p53 ubiquitination. Silencing ANG or inhibiting its nuclear translocation in KSHV+ cells resulted in decreased nuclear LANA-1 and ANG levels, decreased interactions between ANG-LANA-1, ANG-p53 and LANA-1-p53, induction of p53, p21 and Bax proteins, increased cytoplasmic localization of p53, down-regulation of Bcl-2, increased cleavage of caspase-3 and apoptosis of cells. No such effects were observed in KSHV negative BJAB cells. Phosphorylation of p53 was increased in sh-ANG transduced BCBL-1 cells. These studies suggest that the anti-apoptosis observed in KSHV infected cells and suppression of p53 functions could in part be mediated by ANG.

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## Murine Intervertebral Disc ex-vivo Organ Culture: a Novel Model for Genetic and Functional Studies

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**Background**: Despite the individual and societal burdens of degenerative disc disease, the exact genetic etiology and pathogenesis remains elusive. We have developed a novel ex-vivo organ culture model of intact murine intervertebral discs (IVD). Cultured IVDs will provide an excellent, genetically tractable model for further studies on the pathogenesis of degenerative disc disease. **Methods & Results**: IVDs were extracted from wild type, $NF1^{flx/flx}$, and $R26\text{-}hMET^{lsl}$ mice. Explants were cultured in 280-320 mOsm or 380-420 mOsm DMEM supplemented with 10% FBS for 14 days. In wild type mice, quantitative PCR demonstrated maintained *Col1a1, Col2a1*, decreased *Aggrecan*, and increased. *MMP3, ADAMTS4, TIMP1*, and *TIMP2* transcript levels. Trichrome and alcian blue histological sections confirmed structural integrity of cultured and freshly isolated IVDs. To assess cell viability, explants were stained with Propidium Iodide and CFMDA, fixed, embedded and cryo-sectioned. After 14 days in culture, no significant cell death was observed within the annulus fibrosus and nucleus pulposus of the IVDs. Response to exogenous stimuli was assessed by incubating 14 day-cultured IVDs with IL-1 beta. Compared to untreated explant IVDs, IL-1 beta treated IVDs demonstrated large increases in P-Erk and aggrecan breakdown products by immunoblot analysis, with no change in total Erk levels. Explants were incubated with TGF-beta3 and their gene expression profile was assessed by qRT-PCR. *Col1a1, Col2a1, Aggrecan, Timp1*, and *Timp2* expression increased, while *MMP3* and *ADAMTS4* levels decreased. To assess the utility of this model in differing mouse genetic backgrounds, IVDs from $NF1^{flx/flx}$ and $R26\text{-}hMET^{lsl}$ mice were treated with Cre-expressing adenovirus. Allele-specific PCR confirmed successful recombination in 100 percent of $NF1^{flx/flx}$ and $R26\text{-}hMET^{lsl}$ mice explant IVDs. **Conclusion**: Multiple methods have been developed to study the IVD. However, these methods require dissociation of the constituents of the IVD, loss of distinct cell phenotypes, or cannot be genetically manipulated. We have established and characterized a novel ex-vivo murine IVD model. This model offers exciting prospects for further study of the IVD. Further, the ability to genetically manipulate explant IVD *ex vivo* may help to elucidate the complex pathogenesis of degenerative disc disease.

## 158

## Towards Unobstructed Views of the Protein Interaction Landscape

***SJ Pevzner****

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**Background:** The protein interaction landscape remains largely unmapped due to inadequate screening depth, sampling sensitivity issues, and limited assay sensitivity. We screened approximately two-thirds of the human proteome for interactions and compared the resulting unbiased network to other large published interactomes. The provenance of the various networks results in biases related to technology or knowledge, and we develop a framework to quantify such interaction enrichments. The new network exhibits high precision, revealed by validation by multiple orthogonal binary protein interaction assays, and robustness to bias. This robustness results in the detection of previously unknown interactions for many well-studied disease related proteins. We present analyses demonstrating how these new interactions contribute to a more comprehensive understanding of human pathologies.

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## Expression of the BRCA1 Pseudogene in Ovarian Cancer

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**Introduction:** Ovarian cancer is very aggressive and often discovered at a late stage, making it difficult to treat. Although BRCA1 mutations are often linked to inherited ovarian and breast cancers, the majority of cases are due to other genetic alterations. Pseudogenes are originally derived from functional genes but have lost their ability to express proteins due to genetic rearrangements. New evidence suggests that these noncoding genes are potential therapeutic targets in human disease. We have shown that the BRCA1 pseudogene (*BRCA1P1*) has altered expression in breast cancer, which may result in the dysregulation of the cell cycle. Of interest was whether *BRCA1P1* is altered in other cancers, and if it can serve as a diagnostic or therapeutic target in these fatal diseases. **Methods:** We compared expression levels of *BRCA1P1* by qRT-PCR in cell lines from 11 different cancers. We then compared expression of *BRCA1P1* in 4 ovarian cancer (OC) cell lines to immortalized normal epithelial ovarian cells. To study the effect of the pseudogene on proliferation, we used siRNA to target an intron specific to the pseudogene and measured cell proliferation at time points. Luciferase assays were used to measure the promoter activity of *BRCA1P1*. Fluorescent

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In Situ Hybridization (FISH) was used to study the copy numbers of *BRCA1P1* in the OC cell lines. Thirty human ovarian tumor samples and 4 normal ovarian tissue samples were obtained from The University of Chicago Department of Pathology. Expression levels of *BRCA1P1* will be measured and compared to the clinical and pathological data of the tumors. We will use FISH to study copy number variation in the human tumors. **Results:** qRT-PCR results show that *BRCA1P1* is expressed higher in OC cell lines. Promoter activity is also higher in these cell lines compared to normal. Compared to cells transfected with normal control siRNA, those transfected with BRCA1P1 siRNA showed decreased proliferation. Initial FISH studies in OC cell lines suggests an alteration in the copy number of the pseudogene. These experiments will be confirmed in human ovarian tumors. **Conclusions:** The results of our experiments show that the BRCA1 pseudogene is expressed higher in breast cancer and ovarian cancer. This may be the result of altered promoter regulation or copy number variation. We have also shown that the BRCA1P1 may have a role in the regulation of cell proliferation. More research is required to understand how BRCA1P1 is involved in cancer and if it can be used as a diagnostic or therapeutic marker.

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## Exome Sequencing in Familial IgA Nephropathy

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The genetic basis of familial IgA nephropathy (IgAN), a common, immune-mediated cause of kidney failure, is unknown. Familial IgAN segregates as an autosomal dominant trait with incomplete penetrance. We performed exome sequencing in 25 affected individuals from 10 well-characterized IgAN pedigrees. A total of 44Mb or 1.8% of the genome was captured in each individual and subjected to Next-Gen sequencing. In total, 188,211 variants were detected and filtered through a bioinformatics pipeline. We selected variants that were not detected in control exomes and in public databases, had high-quality scores or were shared among affected individuals within the same pedigree. We next prioritized single nucleotide variants (SNVs) that imparted a deleterious effect (nonsense, splice site SNVs, missense SNVs predicted to be damaging by Polyphen2/SIFT, and coding indels). On average, we found 585 novel coding SNVs and indels per individual: 199 were deleterious missense SNVs, 41 nonsense SNVs, 18 SNVs affecting splice sites, and 65 indels. In addition, an average of 75 novel SNVs were shared among patients in each family, with 18 missense mutations predicted to be deleterious, 11 nonsense mutations, and 16 coding indels. However, there were no pathogenic variants shared between families. Thus, out of 188,211 variants, an average of 45 high priority variants were selected for validation in each family. Sanger sequencing of these variants is in progress; validated variants will be further tested for co-segregation with IgAN in additional family members, and their frequency will be assessed in ethnically matched controls. Initial exome sequencing in familial IgAN has identified high priority variants and candidate genes for validation. The absence of independent mutations in the same gene indicates high genetic heterogeneity of this trait, suggesting that a larger sample size may be required to identify shared genetic etiology between families.

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## Large Scale Mitochondrial DNA Deletions Found in Patients with Alzheimer's Disease

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While the consequences of Alzheimer's disease (AD) have become more obvious the cause is still unknown. Recent studies suggest that dysfunctions in the mitochondria of neurons may hold the answer. The goal of this project is to identify and sequence large scale deletions in the mitochondrial genome of neurons from patients with Alzheimer's disease. Cells from the brains of 9 donors (2 without AD, but with PD, 1 with stage 3 AD, 1 with stage 4 AD, 3 with stage 5 AD, and 2 with stage 6 AD) were laser microdissected from 4 different areas (Locus Coeruleus, Substantia Nigra, Hippocampus, and Cerebellum, n=10 each). Adjacent endothelial cells were laser microdissected at the same time to serve as negative controls (n=2 each). DNA from these cells was isolated by column purification and underwent polymerase chain reactions for two large regions (15F-25R and 36F-41R) previously shown to have high mutation rates. Samples with large scale deletions were sequenced at Texas Tech University Sequencing Center and will be sent to Research and Testing for deep sequencing studies. 377 of the 379 samples were shown to successfully amplify at least one of the two control regions. Of the 377 samples, 8 had deletions in 15F-25R region and 13 of the 377 samples had a deletion in the 36F-41R region. All noted deletions (21 in total) occurred exclusively in the hippocampus with an overall rate of 10.3% (21/204 possibilities). 5/7 patients with AD were shown to have deletions in the 15F-25R region with an average deletion rate of 16%, compared to 0/2 without AD but with PD. 1/18 adjacent endothelial cells was positive for deletion. Yet, a correlation between stage of AD and number of deletions could not be established. In the 36F-41R region, the same 5 patients with AD had a deletion rate of 20%. However, deletions were detected in the 2/2 patients without AD but with PD (average rate of 15%). 0/18 adjacent blood vessels showed deletions. Sequencing information has not been analyzed at this time. The observation that large scale deletions were found exclusively in the hippocampus is of interest given its function in formation of new long term memories. Deletions seen in this area in the patients without AD is also unsurprising because these individuals were diagnosed with Lewy Body Dementia, a type of dementia associated with Parkinson's disease. Further analysis may show a link between mitochondrial damage and memory loss.

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## Inhibiting MCU Current in the Heart Causes ATP Deficiency and Metabolic Remodeling

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Mitochondrial $Ca^{2+}$ is a secondary messenger necessary to increase the activity of at least 3 matrix dehydrogenases that enhance oxidative phosphorylation and match cellular energy supply with demand. At the same time, mitochondrial $Ca^{2+}$ overload causes mitochondrial dysfunction, loss of $\Delta\psi$ and myocyte death. Mitochondrial $Ca^{2+}$ overload is a constant feature of heart failure, a major public health problem, but there are no therapies designed to inhibit mitochondrial $Ca^{2+}$ overload. The molecular identity of the mitochondrial calcium uniporter (MCU) was recently identified, allowing us to develop a novel transgenic mouse with cardiac delimited inhibition of MCU current by transgenic expression of a dominant negative MCU (DN-MCU). We hypothesized that DN-MCU mice survive normally into adulthood, but would show physiologic changes consistent with an ATP deficient state. DN-MCU mice were viable and had preserved left ventricular structure and function as assessed by echocardiography. Protein extracts from DN-MCU hearts showed constitutively active AMPK, a master regulator of cellular metabolism that is activated by an increase in the AMP:ATP ratio. We measured $Ca^{2+}$ induced ATP production rates in isolated mitochondria and found that ATP production rates were static in DN-MCU mitochondria. Western blots of whole heart homogenates revealed an increase in GLUT4, LDH and hexokinase. $^{18}$F-fluorodeoxyglucose (FDG) positron emission tomography-computed tomography (PET-CT) imaging revealed higher rates of glucose uptake and metabolism in DN-MCU hearts. $^{11}$C-acetate PET-CT imaging showed reduced clearance of acetate in DN-MCU hearts. 1-$^{13}$C-glucose and 1,2-$^{13}$C-acetate langendorff perfused DN-MCU hearts had higher levels of glycolytic intermediates, including lactate, but lower levels of tricarboxylic acid cycle intermediates than WT hearts. Based on constitutively active AMPK in DN-MCU hearts and less $Ca^{2+}$ induced ATP production in DN-MCU mitochondria, we interpret our results to mean that DN-MCU hearts exist in an ATP deficient state. AMPK, which is activated during mismatch of cellular energy demand and supply, is known to increase rates of glycolytic flux through transcriptional and post-translational mechanisms. Our data show that important glycolytic enzymes are upregulated in DN-MCU mice. Additionally, PET-CT imaging studies and Langendorff perfusion with $^{13}$C metabolic substrates confirm that DN-MCU hearts undergo metabolic remodeling, likely secondary to the inability of DN-MCU mitochondria to increase the rate of ATP formation.

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## Dysfunctional Thrombus Resolution Leading to Abnormal Collagen Fibrillogenesis and Angiogenesis in Injured Arteries of Type III Collagen-Deficient Mice: A Paradoxical Mechanism for 'Tissue Fragility' in Vascular Ehlers-Danlos Syndrome and Spontaneous Cervical Artery Dissection

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Patients harboring mutations in *COL3A1* are predisposed to deadly vascular complications, including spontaneous dissection of cervical arteries and stroke, in association with a sluggish cellular secretion of type III collagen into the extracellular matrix. "Tissue fragility" that presents only episodically as catastrophic rupture of hollow organs in these patients, however, cannot entirely be explained by baseline deficiency of type III collagen in affected tissues. Here we hypothesize that *injury*, and the subsequent remodeling process, is necessary to unmask the vulnerability to "spontaneous" dissection and rupture. We injured cervical elastic arteries in mice by ligation of the left carotid artery, halting proximal blood flow. Strikingly, injured arteries from *Col3a1*$^{+/-}$ mice displayed significant risk for developing thrombi resistant to resolution, relative to the process observed in wild-type littermates, and in contrast to similarities in uninjured right carotid arteries. In a scenario resembling persistent granulation tissue in wound healing, unresolved thrombi in *Col3a1*$^{+/-}$ arteries retain a significantly higher burden of macrophages, proliferative myofibroblasts, and blood-filled defects in lamellar medial layers consistent with penetrating neoangiogenesis. New collagen synthesized by *Col3a1*$^{+/-}$ myofibroblasts in response to arterial injury display irregularly-sized and larger-on-average fibrils, also consistent with matrix abnormalities described in patients suffering from spontaneous cervical artery dissections, even in those without known mutations. Mutant myofibroblasts in 3-D culture models of fibrin remodeling display sluggish expression of *Col3a1* but persistently increasing expression of *Acta2*, relative to wild-type cells whose expression levels peak and subside, following TGF-β1 stimulation. These data implicate a smoldering tissue remodeling in response to injury that increases the risk for angiogenesis and dissection in affected arteries. Finally, immuno-modulation of remodeling by treating injured mice with rapamycin halts thrombus resolution in its earliest phases for both genotypes and similarly prevents the appearance of blood-filled defects as well, suggesting that neoangiogenesis—and perhaps tissue fragility—results from initiating dysfunctional remodeling and not inherent structural weakness commonly assumed to accompany baseline type III collagen deficiency.

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### Early and Late Menarche Associate with Delayed Menstrual Cycles to Predict Metabolic Syndrome 26 Years Later

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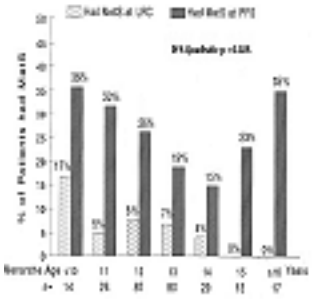
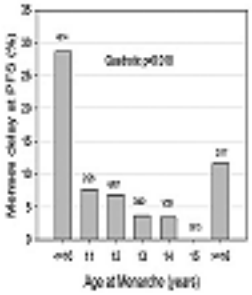
**Objectives:** Determine whether late and early menarche associate with delayed menstrual cycles (≥42 days in adulthood) to predict metabolic syndrome (MetS) 26 years later. **Results:** Early (≤10 years, 5.3% of girls) and late menarche (≥16 years, 6.4% of girls) were both associated with menses delay (≥42 days) in adulthood, 29% and 12%, respectively, vs. 5% for normal menarche, p=0.007. Early menarche was characterized by high childhood BMI (LS mean ± SE 21.3 ±1.0 kg/m$^2$) and high childhood MetS (17%); girls with late menarche had the lowest childhood BMI (18.0 ±1.0) and no childhood MetS. Increasing age at menarche was associated with uniformly decreasing childhood BMI and MetS, but with a U-shaped pattern of MetS (p=0.025) and oligomenorrhea (p=0.018) in adulthood. Change in MetS from median ages 12 to 38 was associated with early-late menarche (OR=2.55, 95% CI 1.11-5.8, p=0.03). MetS in adulthood was associated with childhood MetS (OR=6.62, 95% CI 2.22-19.8, p=0.0007) and with menses delay (OR=3.93, 95% CI 1.46-10.58, p=0.007). **Conclusion:** Early or late age at menarche is a risk factor for both adult oligomenorrhea and MetS. The 12% of girls with early-late menarche (≤age 10, ≥age 16) represent a group at high risk for cardiometabolic abnormalities that is easily identifiable by pediatricians.





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### Association of Insulin Receptor Substrate 1 (IRS1) and JC Virus T-antigen in Colon Cancer

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**Background**: The insulin receptor substrate-1 (IRS-1) protein is an integral part of the IGF-1 signaling pathway. It has been shown to be involved in intestinal epithelial differentiation and is potentially associated with colon cancer progression and liver metastasis. The IGF-1 signaling pathway is dysregulated in many different types of cancer and IRS-1 is involved in the repair of double stranded DNA (dsDNA) breaks through homologous recombination repair (HRR). Sequestration and inactivation of IRS-1 results in the more error-prone non-homologous end joining (NHEJ) repair of dsDNA breaks. One such mechanism of sequestration is binding by the JC polyomavirus protein large T-antigen (TAg). We and others have previously shown that TAg is expressed in colon cancer samples; however, its association with IRS-1 in colon cancer is unknown. Our hypothesis is that the viral protein TAg binds IRS-1 in colonic epithelial cells, thus sequestering IRS-1 and preventing faithful DNA repair through HRR and leading to an increase in DNA mutations. **Materials and Methods**: We obtained 29 formalin-fixed, paraffin-embedded samples including pre-neoplastic polyps and colorectal cancer biopsies. We performed immunohistochemistry and two color immunofluorescence for JCV TAg and IRS-1. We also transfected HCT116 cells with the TAg gene and performed western blot and co-immunoprecipitation to measure IRS-1 levels in the presence of TAg. Finally, we measured the prevalence of HRR in TAg positive and negative cell populations. **Results**: The majority of the colon cancer samples expressed oncogenic TAg (56.7%). Of the TAg positive samples, 15/17 (88.2%) were positive for nuclear IRS-1, whereas only 4/11 (36.4%) of TAg negative samples were positive for nuclear IRS-1 ($p<0.05$). Double labeling immunofluorescence confirmed co-localization of TAg and IRS-1 in the nuclei of neoplastic cells. Moreover, we saw a significant increase in the level of IRS-1 protein in TAg-positive samples, and observed that TAg and IRS-1 co-immunoprecipitate. Finally, we saw that HRR is hindered in cells transfected with TAg. **Conclusions**: JC virus T-antigen protein interacts with and is significantly associated with IRS-1 in colon cancer. This interaction may result in inhibition of high-fidelity homologous recombination repair in favor of the more error-prone non-homologous end joining repair in colon cancer, resulting in the accumulation of genetic mutations.

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### Residue Changes in a La Reunion Strain of Chikungunya Virus are Responsible for Increased Disease Severity in Neonatal Mice

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Chikungunya virus (CHIKV) is an emerging arbovirus that has infected millions of people in Africa and Asia and has the potential to spread worldwide. Here we compare two strains, a West African strain (37997) and a strain from the 2006 La Reunion outbreak (LR2006 OPY1) to determine if residue differences between the strains could explain the severity of the recent outbreaks. Although there were no differences between the two strains in terms of *in-vitro* growth kinetics or mortality in BL6 pups, several differences were seen in viral titers of organs. First, there was a three-day delay of clearance of the LR2006 OPY1 strain from serum in comparison to the 37997 strain. Additionally, the LR2006 OPY1 strain produced viral titers in muscle several logs higher than the 37997 strain at the peak of infection. Muscle taken from pups infected with either strain exhibits necrosis and degeneration, however those pups infected with the LR2006 OPY1 strain have a more widespread and severe phenotype. Both strains were able to induce similar production of cytokines, chemokines and neutralizing antibodies

in pups. Together, this data suggests that muscle damage during CHIKV infection is controlled by a viral determinant in the genome of the LR2006 OPY1 strain. A gene of interest will be identified by creating chimeric viruses in which a gene of LR2006 will be swapped for the same gene on the 37997 background. These viruses will be screened in pups for muscle and serum titers.

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## Interferon-Induced Transmembrane (IFITM) Proteins are Upregulated in Human Platelets: Novel Immune Sensing of H1N1 Influenza and Other Pathogens

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**Background:** IFITM proteins restrict influenza viral replication and are integral to mediating influenza infections. As platelets are emerging as versatile immune effector cells, we hypothesized that platelet IFITM expression would be significantly altered in human subjects infected with the 2009 H1N1 (Swine Flu) influenza virus. **Methods:** We first performed paired-end next-generation RNA sequencing (RNA-Seq) in platelets isolated from acutely infected patients and matched healthy controls, which has not been previously reported. Differentially expressed candidate mRNAs were identified. We then characterized IFITM mRNA and protein in platelets isolated from critically-ill patients with bacterial sepsis (n=25) or confirmed H1N1 influenza infection (n=24) and healthy control subjects (n=31). Plasma levels of interferon(IFN)-γ were measured via Luminex. To examine platelet IFITM expression in humans under non-infected conditions, we measured platelet IFITM in healthy subjects following influenza vaccination. In separate experiments, CD34-derived megakaryocytes were stimulated with IFN-γ and we measured IFITM mRNA and protein expression. **Results:** RNA-Seq performed on platelets from infected patients demonstrated that numerous transcripts (>1,000) are dramatically altered compared to controls. In particular, *IFITM-1*, *-2*, and *-3* increased robustly. In platelets from infected patients, IFITM-3 mRNA and protein was significantly upregulated (25 to 100-fold). Influenza patients had the greatest increases in platelet *IFITM-3* mRNA and protein. Plasma IFN-γ levels were also increased in influenza patients. Mortality was higher in influenza patients with blunted platelet *IFITM* responses. Influenza vaccination in healthy subjects induced platelet *IFITM* mRNA and protein expression, indicating that platelets and/or megakaryocytes receive immune signals that alter *IFITM* expression. In vitro, *IFITM* mRNA and protein was induced in IFN-γ stimulated megakaryocytes, but not in IFN-γ stimulated platelets from healthy controls. **Conclusions:** These findings provide novel biological evidence that platelets undergo dynamic changes in their molecular signature during infectious syndromes. IFITM proteins are markedly upregulated in platelets from infected patients and in healthy human subjects following influenza vaccination. Influenza patients had the greatest increase in platelet IFITM, consistent with IFITM-mediated restriction of influenza viral replication. We postulate that platelet IFITM induction may occur in a signal-dependent mechanism mediated through megakaryocytes, perhaps during interactions with viruses or viral particles in the lung or via systemic inflammatory pathways.

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## Nephrocystin-5 Knockout Mouse is a Novel Model of Senior-Løken Syndrome Recapitulating Both Retina and Renal Pathologies

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Senior-Løken Syndrome (SLSN) is a rare autosomal recessive disease characterized by both progressive retinal degeneration (Retinitis Pigmentosa/Leber congenital amaurosis) and medullary cystic kidney disease (nephrononophthisis). SLSN is part of a larger disease class, called 'ciliopathies,' in which the defect lies in proteins localized to the primary cilium or photoreceptor connecting cilium. A class of heterogeneous genes is now known to cause SLSN and associated syndromes (NPHP1-13). Defects in Nephrocystin-5 (*NPHP5/IQCB1*) most frequently cause Senior-Løken Syndrome. This 598 amino acid protein is expressed in the primary cilium of most cell types; however, the function of NPHP5 is poorly understood. Here, we describe a mammalian model of Senior-Løken syndrome generated by deleting *Nphp5* in the mouse. Global knockout of *Nphp5* produces viable and fertile mice. Retinal function (specifically rod and cone photoreceptor function) in knockout animals was undetectable by electroretinography (ERG) at postnatal day 12 (P12), just after eye opening. This is explained by absence of outer segments, the photosensitive part of photoreceptors normally containing rhodopsin, at P10. Overall photoreceptor cell number is also decreased significantly by P10. Rhodopsin transport is impaired as early as P6, accumulating in rod perinuclear regions, which may account for rapid rod degeneration. The kidneys of knockout animals show increased apoptosis, fibrosis and presence of cysts, which is reminiscent of the pathology in humans. Knockdown of *Nphp5* in a mouse kidney cell line shows decreased numbers of primary cilia suggesting a role of NPHP5 in ciliogenesis or ciliary maintenance in the target organs. Ultrastructure of the photoreceptor connecting cilium and basal body was examined on postnatal days 6 and 10 to assess the ciliary and axonemal compartments. The NPHP5 global knockout mouse is a novel model of Senior-Løken syndrome recapitulating the retina and renal pathologies observed in humans. This model will be an important tool to study NPHP5 function and molecular mechanisms leading to retinal and renal dystrophies.

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## Control of Innate Antiviral Immunity by HIV-1

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Cell-intrinsic innate immune defenses are essential for the control of virus infection and can serve to restrict HIV-1 replication and spread during acute infection. Interferon regulatory factor (IRF)-3 is a central transcription factor of innate immune signaling that induces the expression of antiviral and immunomodulatory genes whose products can respectively suppress HIV-1 infection

within CD4+ T cells/macrophages and regulate the adaptive immune response to infection. We have found that during acute mucosal infection, and infection of cultured cells *in vitro*, HIV-1 evades innate antiviral immunity through the actions of the Vpu protein, which binds IRF-3 and mediates its redistribution into the host cell lysosome for proteolytic destruction. Thus, we hypothesize that IRF-3 degradation by Vpu serves to support HIV-1 replication and dissemination and to dysregulate the adaptive immune response. Vpu-mediated inhibition of IRF-3 function abrogates the virus-induced expression of known HIV-1 restriction factors including APOBEC3G, BST2, and other interferon-stimulated genes of host defense, resulting in enhanced cellular permissiveness to infection. Conversely, we found that in the absence of Vpu HIV-1 infection induces robust expression of IRF-3-dependent genes that function to suppress viral replication. To define the molecular mechanisms of Vpu interaction with IRF-3, we assessed the interaction with Vpu of IRF-3 truncation mutants. Co-immunoprecipitation analyses revealed that the IRF-3 binding epitope for Vpu is an 88-amino acid region within the IRF Association Domain (IAD). We predict that Vpu binding to the IAD will disrupt the formation of active IRF-3 dimers. We further predict that IRF-3 depletion and control of innate antiviral immunity by HIV-1 may correlate with disease progression in HIV-infected patients. To this end, we have developed two novel monoclonal antibodies to human IRF-3 to support the study of IRF-3 activation and HIV-mediated IRF-3 depletion among patient samples in a high-throughput manner. One of these antibodies, AR-1, is specific for activated IRF-3. The other, AR-2, detects total IRF-3 levels in a flow cytometric assay of blood leukocytes. Use of these new antibodies to study IRF-3 levels during HIV infection could reveal an innate immune correlate of HIV-1 disease progression, while studies to fully define the interaction between Vpu and IRF-3 may reveal novel targets for the development of drugs that preserve IRF-3 activity during HIV-1 infection.

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## c-Src Regulation of Connexin43 Remodeling in Ischemic Heart Disease

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**Background**: Ischemic heart disease (IHD) is associated with decreased cardiac conduction, providing substrate for reentrant arrhythmia and sudden cardiac death. Connexin43 (Cx43) is the principal gap junction protein responsible for current propagations through cardiomyocytes in the ventricles. Cx43 expression is known to be reduced in the left ventricle following IHD. Recently, we showed that ROS stimulates c-Src phosphorylation (p-Src), allowing p-Src binding to the scaffolding protein zonula occludens-1 (ZO-1) and destabilizing Cx43, leading to Cx43 lateralization and degradation. We tested whether Src inhibition would prevent Cx43 degradation in IHD. **Methods:** Coronary artery occlusion was performed on 12-week-old mice causing myocardial infarction (MI). MI mice were treated with PP1, a p-Src inhibitor, or PP3, an inactive analogue. PP1, PP3, and sham hearts were compared functionally by echocardiography, optical mapping, ECG telemetry analysis, and arrhythmia inducibility by ventricular pacing. Tissues were collected for immunohistochemistry and Western blot analysis. Additionally, Cx43 restoration was evaluated following treatment with Saracatinib, a clinically relevant p-Src inhibitor. **Results:** PP1 treated groups demonstrated restored conduction velocity when compared to PP3 treated mice (PP1=33 cm/s, PP3=18cm/s) and lowered the incidence of inducible arrhythmia (71% of PP3 mice, 35% of PP1 mice). In PP1-treated mice, there was a 60 % decrease in p-Src activation and a 25% increase in Cx43 expression at the scar border compared to PP3 groups. PP1 did not change infarct size, ECG pattern, or cardiac function (Ejection Fraction: Sham=61±1, PP1=39±2, PP3=39±2). Saracatinib treatment demonstrated restoration of Cx43 and p-Src inhibition comparable to PP1. **Conclusions**: Src inhibition improves Cx43 levels and conduction velocity after MI. Src inhibitors may represent a new class of antiarrhythmic compounds for treatment following MI.

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## Strain-like Templating of α-Synuclein Inclusion Pathology in Neurons and Astrocytes

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Genetic studies have established a causative role for α-synuclein in PD, and the presence of α-synuclein aggregates in the form of Lewy body (LB) and Lewy neurite (LN) protein inclusions are defining pathological features of Parkinson's disease (PD). Recent data has established that extracellular α-synuclein aggregates can induce intracellular α-synuclein pathology, and supported the hypothesis that α-synuclein pathology can spread via a prion-like self-templating mechanism. Here, we investigated the potential for strain-like seeding using recombinant wild type and PD-linked mutant (A53T and E46K) α-synuclein aggregates in primary mixed neuronal-glial cultures. We find that wild type and A53T α-synuclein fibrils seed flame like inclusions in both neurons and astrocytes in primary neuronal cultures, whereas the structurally distinct E46K fibrils seed punctate, rounded inclusions. Notably, these differences in seeded inclusion formation in culture reflect differences in inclusion pathology seen in transgenic mice expressing the A53T or E46K α-synuclein mutants. We further show that the inclusion morphology is dictated more by the seed applied than the form of α-synuclein expressed. These studies establish for the first time that templating of α-synuclein inclusion pathology exhibits a prion-like strain dependence in cells, supporting a self-templating mechanism of inclusion formation in these model systems.

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## CdTe Quantum Dots Activate Human Platelets: Potential Implication for Nanoparticle Hemocompatibility

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**Background:** Nanomedicine approaches, many of which utilize engineered nanoparticles, have raised concerns regarding their biocompatibility. Semiconductor nanocrystal quantum dots (QDs), owing to their unique optoelectronic properties, are promising candidates for incorporation in the nanotechnology based diagnostic platforms. Systemic administration of these nanotools has the potential to interact with cellular components of blood. Platelets are anucleate cell elements derived from megakaryocytes that play a crucial role in haemostasis and thrombosis. **Objectives:** The aim of this study was to systematically investigate the effect of cadmium telluride QDs on human platelets. **Methods:** Platelet rich plasma and washed platelets were isolated from human blood. Platelet function studies were carried out under quasi-dynamic conditions utilizing light transmission aggregometry, flow cytometry, immunofluorescence studies and gelatin zymography. Quartz crystal microbalance with Dissipation (QCM-D) was employed to measure QD-induced platelet microaggregation under flow conditions. Morphology of platelet aggregates were analyzed by phase contrast, atomic force and transmission electron microscopy. **Results:** CdTe QDs can activate human platelets by binding to platelet plasma membrane via up-regulation of GPIIb-IIIa and P-selectin receptors, and release of matrix metalloproteinase (MMP)-2. These findings present the first data on the mechanism of functional response of blood components to ultra-small NPs under experimental conditions closely imitating *in vivo* scenarios.

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## Endothelial PGC-1alpha Mediates Vascular Dysfunction in Diabetes

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Endothelial dysfunction is a central hallmark of diabetes. The transcriptional coactivator PGC-1alpha is a powerful regulator of metabolism in numerous cell types, but its role in endothelial cells remains poorly understood. We show here that hyperglycemia induces the expression of PGC-1alpha in endothelial cells in cell culture and *in vivo*, and that PGC-1alpha powerfully blocks endothelial migration in cell culture and vasculogenesis *in vivo*. Conversely, VEGF and other pro-angiogenic stimuli rapidly downregulate PGC-1alpha, and deletion of PGC-1alpha phenocopies the pro-migratory effect of VEGF. Mechanistically, PGC-1alpha blunts activation of Rac/Akt/eNOS signaling in response to VEGF or sphingosine 1-phosphate (S1P), established activators of endothelial cells, while leaving the ERK arm intact. Transgenic overexpression of PGC-1alpha in endothelial cells in mice mimics multiple diabetic phenotypes, including aberrant re-endothelialization in response to carotid injury, blunted wound healing, and reduced blood flow recovery in response to hindlimb ischemia. Conversely, deletion of PGC-1alpha in endothelial cells rescues wound healing dysfunction in type I and II diabetic animals, and rescues blood flow recovery in type I diabetic animals with hindlimb ischemia. PGC-1alpha thus potently inhibits endothelial function andangiogenesis, and induction of PGC-1alpha by hyperglycemia contributes to multiple aspects of vascular dysfunction in diabetes.

## 174

## Strengthening Physician-scientist Training Through Journal Clubs: Revitalizing an Underutilized Tool

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The Medical Scientist Training Program (MSTP) of the University of Cincinnati (UC) has redesigned its journal clubs to emphasize a learner-centered format with defined educational objectives. This redesign was prompted by the challenge of creating a meaningful educational experience for a community of learners with substantial diversity in clinical and research experience, and varied academic focus. Initial reform efforts targeted the optional summer journal club as an opportunity to create program identity during this first interaction among incoming students and the UC-MSTP. Clinical and translational research topics were chosen over traditional basic science topics, due to their greater applicability and accessibility to all attendees. Pre-selected learning topics and learning objectives were presented to weekly rotating student leaders, who identified a relevant research article with input from selected faculty experts. Sessions opened with a brief didactic presentation from student leaders, which focused on learning objectives for the week, followed by group interpretation and discussion of a related research article. During the first cycle in 2011, students' self-rated knowledge of all 17 assessed learning objectives increased, and attendance at these voluntary sessions tripled from previous years. In the 2012 cycle, students also completed objective knowledge assessments. Scores on this assessment were significantly higher following the program, and 100% of students showed improvement in their overall assessment scores. Students expressed high satisfaction following both cycles. The program was also successful at creating vertical interaction between students; the majority of MSTP students chose to participate in this voluntary activity, and those that participated in 2012 reported an increase in the number of other UC-MSTP students they could talk to about a professional or personal problem, although this did not reach statistical significance. Unstructured student feedback indicated that the program's success could be largely attributed to the mixed format of introductory didactics, group discussion and faculty content expert input into the

selection of relevant articles. Due to the success of this model, the UC-MSTP is now reforming the journal clubs during the academic year, using a similar structure to address basic science techniques in fall 2012. In addition, MSTP student leaders have worked with UC medical education faculty to adapt exercises originally designed for this forum to the general medical curriculum. Two such exercises on clinical and translational topics have already been implemented for all preclinical medical students at UC.

## 175

## Exploring Stemness and Hypoxia in Osteosarcoma; Connecting Hypoxia-inducible Factors to Tumor-initiating Cells

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**Background**: Osteosarcoma (OS) is the most common type of solid bone cancer, mainly arising in children and young adults. Cell populations bearing a stem cell-like phenotype have consistently been isolated from both primary and metastatic OS lesions. These cells are thought of as tumor initiating (TICs) in that they display characteristics of self-renewal and multipotency while also demonstrating chemoresistance and increased propensity for metastasis. Tumor hypoxia and hypoxia inducible factors (HIF) influenced the acquisition or maintenance of a stem cell-like phenotype in other solid human cancers. Our objective is to demonstrate the effect of HIF signaling in the acquisition and maintenance of stemness in human osteosarcoma. **Methods/ Results**: We used osteosarcoma cell lines (mHOS, 143B, MG-63) and primary human osteosarcoma cell isolates for experimental analysis. Cells were incubated in a hypoxia chamber set to 2% $O_2$ for 24, 48, and 72 hours. We observed upregulation of downstream targets of HIF (VEGFA, GLUT1, PFKF-P, OCT-4) using q-rtPCR, and stability of HIF proteins using western blot. We observed in-vivo OS tibial xenografts showing focal HIF protein expression around areas of necrosis and at tumor borders via immunohistochemistry. Furthermore, we can grow OS cell lines and patient-derived samples in anchorage-independent conditions as spherical clusters (sarcospheres). These spheres demonstrate increases in stem cell related gene expression via q-rtPCR, including Oct-4, Sox-2, and Axin2 as a measure of Wnt signaling. These spheres can be passaged showing enrichment of stem cell gene expression and display chemoresistant properties to cisplatin in the 1-5μM range. **Conclusions**: HIFS play an important role in the adaptation to hypoxia of OS cells. OS cells resembling the phenotypic characteristics of TICs can be isolated through growth in anchorage-independent conditions. Current experiments include quantifying sphere formation under either hypoxic or normoxic conditions, and the development of a genetically engineered mouse model of osteosarcoma via dysregulation of p53 and Vhl in osteoblasts to study the role of HIFs on osteosarcoma tumor progression.

## 176

## Positively Selected FimH Residues Confer Enhanced Virulence for Uropathogenic *Escherichia coli*

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Urinary tract infections (UTI) affect 50% of women at least once during their lifetime. Up to 40% of these women suffer recurrent UTI, leading to increased use of antibiotics, which promotes rising resistance. This vicious cycle can lead to chronic UTI by prolonging bacteriuria and symptoms. The most common cause, uropathogenic *E. coli* (UPEC) invades bladder tissue and establishes intracellular bacterial communities (IBCs) in both mice and humans. UPEC binding to epithelial cells and subsequent invasion relies on the FimH adhesin, which binds mannosylated residues called uroplakins on the bladder surface. This invasive cycle allows UPEC to subvert immune defenses and penetrate a severe population bottleneck. Specific residues in FimH are under positive selection among UPEC compared to fecal *E. coli*. We determined the effect of these residues on acute and chronic infection caused by two prototypical UPEC strains: UTI89, a cystitis isolate, and CFT073, a urosepsis isolate. The whole genomes of UTI89 and CFT073 are 99% identical; however, FimH has two differences in positively selected residues. Interestingly, these differences are remote from the mannose binding pocket, suggesting they do not directly alter affinity to mannose. To test the effects of these differences, we swapped fimH alleles between UTI89 and CFT073 and assessed their pathogenesis in mouse models. We found that harboring FimH from UTI89 in both strains increased IBC number and the propensity to cause chronic cystitis in single infections. During chronic infection, UTI89 FimH dramatically outcompeted CFT073 FimH as early as one day post infection in urine. In the bladder at sacrifice, strains harboring UTI89 FimH were 100,000 more abundant than strains with CFT073 FimH. We show that UTI89 FimH confers enhanced binding to bladder tissue as well as increased invasion and IBC maturation. These data show that FimH is crucial in modulating UPEC virulence and may suggest a prognostic test to predict the potential severity of a UTI based on FimH sequence.

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## Preventing Mature Biofilm Formation on Colonized Surfaces Using Antibiotics

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**Background:** Microbial biofilm formation on implanted medical devices is an important contributor to nosocomial infections. We are interested in determining whether there is a dose of antibiotic that could prevent the development of mature biofilms following

surface colonization of a medical device. We hypothesized that a preventative antibiotic concentration would increase as a function of the microbial areal density (number of cells per $mm^2$). Our initial research seeks to characterize the relationship between the areal cell density of *Bacillus subtilis* and the amount of ampicillin required to prevent biofilm growth. To do so, overnight culture of *B. subtilis* were recirculated through a flow cell in order to attach specific numbers of cells, followed by 24 hour continuous flow of Luria-Bertani broth containing ampicillin at varying concentrations. After the growth period, the biofilms were stained and imaged by confocal laser scanning microscopy and the resultant images were digitally quantified. Results from the 2 hour recirculation indicate a linear relationship between the concentration of cells recirculated and the number of cells that attached to the flow cell surface. Preliminary results indicate that a concentration of 25 ppm ampicillin is sufficient to inhibit growth of *B. subtilis* at both 600 and 1000 cell per field. Changes in biofilm structure and cell morphology were evident in response to increasing ampicillin concentrations. These findings will be examined further in future work and expanded to other species of bacteria.

## 178

## Investigation of Gene Networks and Their Role in Motor Programs

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**Background:** Processes such as breathing, locomotion, and ingestion require a well-connected network of cells that are in turn regulated by a network of genes. The nematode *C. elegans* allows us to integrate the study of the gene and cellular networks associated with locomotion. The cellular network that we are investigating involves a cross-inhibitory network composed of the DD and VD motor neurons (collectively termed the D motor neurons (mns)) and the muscles they innervate. The D mns contribute to the animal's sinuous pattern of locomotion by causing muscle relaxation. The gene network includes two transcription factors UNC-30 and ALR-1 and a large number of genes involved in the anatomical and physiological characteristics of the D mns. We have used two approaches to analyze the relationships between the gene and cellular networks: bioinformatics and genetics. In the bioinformatics approach, potential transcription factor binding sites in the upstream regulatory region of a neuropeptide gene in the D mns, *flp-11,* were analyzed using software, such as MUSSA and TESS. The two candidates that emerged were then analyzed using a genetic approach. We reasoned that if these two transcription factors regulated *flp-11*, then the pattern of a *pflp-11::gfp* reporter would be altered in a mutant background. *pflp-11::gfp* was crossed in an *alr-1* and an *unc-30* mutant backgrounds. The normal *pflp-11::gfp* pattern expression was observed in an *unc-30* mutant background, but not in an *alr-1* mutant background. In future studies, we will continue to bind these two approaches to study the relationship between gene and cellular networks.

## 179

## Intravascular Neuromodulation Using a Catheter Based Electrode

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The ability to control neural structures from a vascular position would create a paradigm shift in the diagnosis and treatment of neurological diseases such as seizures, movement disorders, and mental illness. In this study we explore the possibility of safely using an electrode-tipped catheter to generate pulsed electrical fields that modulate neural structures adjacent to a given vessel. Previous data shows that the minimal field strength needed to synchronize neural network activity is 0.1 – 0.2 V/m. Neural stimulation requires supra-threshold field strengths of 15-20 V/m. Ablation of neural structures via electroporation is slightly higher at approximately 500 V/m. These values are much lower than those needed to cause any vascular effects. Studies show that vasoconstriction requires at least 2000 V/m and thrombosis requires at least 5500 V/m for 30min. No thermal damage has been detected at the threshold levels for neuromodulation. The established sensitivity of neural structures to weak external electric fields allow for engineered devices to achieve minimally invasive neuromodulation from within the vasculature. We have developed a prototype design for such a device.

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## Do Circulating Endothelial Cells Microparticles (EC-MPs) Alter the Peripheral Blood Derived Monocytes (PBDMs) Efferocytosis of Smokers and COPD Individuals?

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**Rationale:** Cigarette smoking leads to COPD with its phenotypes of chronic bronchitis and emphysema, and to various systemic co-morbidities, attributed to a systemic inflammatory state. Alpha-1 antitrypsin (A1AT) deficiency increases the COPD risk, rendering A1AT as a protective factor against emphysema. In emphysema apoptosis of lung endothelial cells (EC) results in alveolar-capillary membrane destruction. Recently, EC-MPs have been detected in the plasma of emphysema patients, which suggests increased release from apoptotic EC and delayed clearance by phagocytes such as PBDMs or macrophages. The role of EC-MPs in COPD is currently unknown. We hypothesized that EC-MPs clearance by PBDMs may be delayed in COPD and that EC-MPs may themselves impair PBDMs efferocytosis, in a process that could be ameliorated by A1AT. **Methods:** Blood samples were collected from non-smokers, non-diseased smokers, and individuals with COPD. EC-MPs were isolated from plasma by ultra-centrifugation at 100,000g (2 h, 4C). Labeled EC-MPs ($CD31^+/CD42b^-$) were counted by flow-cytometry. Functional PDBMs were isolated using the Dynabeads Untouched Human Monocytes kit. NR8383 cell line was also used in select efferocytosis experiments. Nile-red labeled EC-MPs were incubated with A1AT-treated (100ug/mL, 4h and 24h) or untreated PBDMs which were tested in

phagocytosis assays with CTO-labeled, apoptotic Jurkat cells using flow-cytometry. Results: Non-diseased smokers and COPD individuals had increased number of EC-MPs compared with controls. As expected, their PBDMs demonstrate decreased efferocytosis compared with controls. Treatment with A1AT (4h) further decreased PBDMs efferocytosis; however prolonged administration (24h) rescued smoker's PBDMs efferocytosis. Co-culture of EC-MPs isolated from smokers / NR8383 macrophages impaired engulfment of apoptotic targets, which was ameliorated by A1AT(4h). We investigated TACE (ADAM17) a sheddase for the efferocytosis receptors, as possible mechanism of A1AT action. TACE activity in the membrane fractions was significantly inhibited by A1AT. **Conclusions:** The increased EC-MPs in plasma of smokers and COPD individuals may not be merely biomarkers of endothelial damage, but may have an inhibitory effect on PBMCs efferocytosis, which could enhance systemic inflammation. Prolonged A1AT treatment enhanced PBMCs efferocytosis, possibly by maintaining scavenging receptor function at the plasma membrane.

## 182

## Genome-scale Expression Screen Reveals Role for YAP1 in KRAS Oncogenic Addiction

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Cancer cells harboring mutations in *KRAS*, the most commonly mutated oncogene in cancer, exhibit dependency on continued expression of the KRAS protein. Despite extensive studies of KRAS effectors, there are gaps in understanding the relevant biological pathways underlying this dependency, a phenomenon termed oncogene addiction. To look for genes that underlie KRAS oncogenic addiction, we performed a genome-scale expression screen of ~15,000 open reading frames (ORFs) to identify ORFs that are able to maintain cell viability in even after KRAS suppression. The transcriptional co-activator *YAP1* scored as the strongest candidate. We validated the functional relationship between YAP1 and KRAS signaling in cell lines and in *KRAS*-driven transformation models *in vitro*. Furthermore, we observed increased YAP1 signaling *in vivo* in a mouse model of acquired KRAS resistance. We used an unbiased expression profiling approach to determine downstream targets of YAP relevant for KRAS oncogenic addiction. Our results identify a novel convergence of the KRAS and YAP pathways at the transcriptional level. Moreover, our results suggest that oncogenic addiction to KRAS can be overcome by parallel pathways that converge on critical downstream effectors, analogous to models of resistance to targeted kinase therapies in the clinic.

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## Retinal Pigment Epithelium Cells Show a Time-dependent Increase in Soluble Betacellulin Expression in Response to Extracellular Insulin

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**Background:** Soluble betacellulin (s-BTC), a 19 kDa member of the epidermal growth factor family, has been suggested to play a role in the pathogenesis of diabetic macular edema. It has previously been shown that s-BTC is increased in retinas of diabetic mice and humans, and that s-BTC induces increased retinal vascular permeability *in vivo*. However, factors which regulate s-BTC in diabetes are currently unknown. In addition, it is still unclear whether the increased s-BTC seen in diabetic retinas is due to local (e.g., from retinal pigment epithelium cells lying below the retina) or systemic production. **Methods:** ARPE-19 cells (a retinal pigment epithelium (RPE) cell line) were grown in culture, serum-starved for 24 hours, and then exposed to 10 nM regular human insulin. Cells were harvested by mechanical lysis at 5 minutes, 15 minutes, 30 minutes, 1 hour, 12 hours, 24 hours, and 48 hours. Lysates were then analyzed by western blot for expression of s-BTC as well as two members of the insulin signaling pathway, phosphorylated p38 and phosphorylated ERK1. **Results:** In RPE cells treated with insulin, s-BTC expression was detectably increased at 12 hours, showed a peak at 24 hours, and remained moderately elevated at 48 hours compared to RPE cells treated with no insulin. Phosphorylated p38 expression peaked at 1 hour of insulin treatment, and returned to non-insulin treated levels at 48 hours. Phosphorylated ERK1 expression peaked between 30 minutes to 1 hour of insulin treatment, and remained slightly elevated at 48 hours compared to non-insulin treated cells. **Conclusion:** RPE cells may contribute to retinal s-BTC levels *in vivo* in diabetic macular edema, and their production of s-BTC may be regulated by extracellular insulin levels. In RPE cells treated with insulin, both phosphorylated p38 and phosphorylated ERK1 expression peak prior to elevation of s-BTC level. The expression of all three proteins peak and decrease within 48 hours of insulin treatment. Further work needs to be done to investigate whether phosphorylated p38 or phosphorylated ERK1act as intermediates between insulin and s-BTC expression, and whether RPE cells can become sensitized or desensitized to extracellular insulin.

## 184

## Cross-species Synthetic Lethal Screening to Identify Novel Therapeutic Targets in Cancer

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Synthetic lethal interactions are a type of genetic interaction in which loss of function of two genes in combination results in cell death. Recently, there has been much interest in the discovery of drugs that are selectively toxic to cancer cells by targeting proteins that form synthetic lethal interactions with tumor-suppressor genes. The demonstrated clinical activity of Poly (ADP-ribose) polymerase (PARP) inhibitors in early phase clinical trials

has demonstrated the clinical viability of this strategy. Synthetic lethal interactions can be quantified and measured systematically using a simple read-out such as cell viability to create interaction networks known as Epistatic Miniarray Profiles, or E-MAPs. Harnessing the power of yeast genetics, in the model organism *S. Cerevisiae* we have conducted an epistatic interaction screen including 162 orthologs of known human tumor suppressor genes and 596 genes representing the orthologs of all human 'druggable' genes as well as genes known to participate in the DNA damage response. This screen has produced over 120,000 quantitative interaction measurements (tests of interaction for individual gene pairs) assayed in the presence and absence of DNA damaging chemotherapy. A computational algorithm was developed to prioritize the interactions with greatest probability of conservation in humans based on conservation in the fission yeast *S. pombe*, and the presence of synthetic lethal interactions amongst neighboring genes in the protein interaction network. Among the highest scoring interactions was that between RAD51 (ortholog to human BRCA1) and HDA1 (ortholog to human histone deacetylases (HDAC)). The cross-species conservation of the synthetic lethal interaction of RAD51 and HDA1 in *S. Cerevisiae* was tested in human cancer cell lines. In LN428 glioblastoma multiforme cells the pan-HDAC inhibitor vorinostat was significantly more toxic to BRCA1-knock down cells compared to wild-type controls. In UWB1.289 BRCA1 mutant ovarian cancer cells, restoration of wild-type BRCA1expression decreased the potency of vorinostat. Interestingly, the addition of either DNA damaging chemotherapy or a PARP inhibitor potentiates this effect. These results demonstrate for the first time the cross-species conservation of a synthetic lethal interaction between yeast and humans, validating high-throughput screening in yeast as a strategy to identify novel therapeutic targets in human cancer.

## 185

## Inferring Regulatory Mechanisms from Stochastic Signatures in Gene Expression

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Recent cancer genome studies report that mutations arise in a vast number of different genes and occur in both coding and non-coding regions. A key obstacle to translating these observations into individualized treatment is distinguishing functional mutations from normal human variation, and understanding how the functional variants disrupt gene expression. Computational models which use the rate constants that govern gene expression to predict protein levels show promise in determining the function of novel mutations. A key challenge in usefully deploying these models is determining these rate constants *in vivo*. To address this challenge we developed an approach for inferring mechanistic, molecular-level information about gene expression from flow cytometry data. Using a stochastic model written in terms of the rate constants for gene accessibility, transcription and translation rates, and RNA and protein degradation rates, we are able to capture the full shape of a protein concentration distribution, an improvement over current methods which model only the mean of these distributions. To test our method, we simulated 7968 gene expression distributions representing all physiologically reasonable parameterizations. We find that our model correctly predicts some or all of the underlying rate constants in 91% of our test distributions. Application of our method *in vivo* will allow investigators to determine which rate constants are perturbed by particular disease causing mutations. We are confident that bringing to bear more quantitative, mechanistic approaches to understanding gene expression will help pave the way for personalized medicine.

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## Epidemiological Profile of Patients with EVA in a Seven Year Period in an ICU for Adults

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**Objective:** Describe the epidemiological profile of patients with encephalic vascular accident (EVA) admitted in an intensive care unit (ICU) in Brasília-Brazil, in order to characterize the distribution of different EVA subtypes, their clinical evolution and complications. **Methods:** It was performed a prospective analysis of 324 medical records of patients who were diagnosed with EVA and were admitted to the ICU of the Hospital Santa Lucia, Brasília, DF, Brazil, from October 2004 to December 2010. **Results:** Among the patients analyzed, 50% were male and 50% female with a mean age of 64.94 years old, which 60.80% were affected by ischemic EVA, 38.27% by hemorrhagic stroke with and 1% not rated. Among the incidences of the risk factors were found: systemic arterial hypertension with 65.74% of the cases; diabetes mellitus, 26.85%; dyslipidemia, 25%, and family history in 22.53% of cases. About the complications, respiratory failure was observed in 21.9% of cases, aspiration pneumonia in 16.66% and 9.56% infection. The death rate was 21.60%. **Conclusion:** The infection rate of 9.65% was lower than the average rate found in the literature, however, the other epidemiological aspects and complications analyzed classically described reproduced the pattern, replicating the common challenge for intensive care services due to the high potential for morbidity and mortality of these patients associated with this diagnosis.

# Poster Abstracts

## 188

### An *in vitro* Model of Ovulation and Luteinization Identifies Green Tea EGCG as a Possible Contraceptive

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Ovulation is the capstone of the reproductive cycle, resulting in egg expulsion and somatic cell luteinization. Periovulatory events establish a woman's fertile window and serve as important targets for contraception. The present study sought to investigate periovulatory mechanisms using a 3D hydrogel culture system. First, to establish an *in vitro* assay for follicular rupture, murine follicles were cultured in alginate, treated with hCG for 14 hours, and corresponding oocytes were scored for meiotic competence. Post-hCG 95% of follicles ruptured and of the follicles that ruptured, 91% released meiotically competent (MII) eggs. The proteases required for ovulation remain undefined, thus follicles were concomitantly treated with inhibitors and hCG to assess the role of individual enzymes in follicular wall breakdown. Follicles treated with leupeptin (serine protease inhibitor) ruptured at an equivalent rate as the control group. However, follicles treated with SB-3CT (MMP-2/9 inhibitor) and RU-486 (PR antagonist, FDA-approved emergency contraceptive) ruptured at significantly lower rates (39% and 43%, respectively). This suggests that MMP-2/9 are important for follicular wall breakdown. In addition, epigallocatechin gallate (EGCG), the most abundant anti-oxidant in green tea with anti-protease activity, significantly inhibited follicular rupture (16%) and COC expansion. To assess the contraceptive effects of EGCG *in vivo*, EGCG was injected into the ovarian bursa and saline injected on the contralateral side. 16 hours post-hCG trigger, all of the ovaries (n=4) treated with EGCG contained unruptured luteinized follicles with trapped eggs, while sham-treated ovaries contained corpora lutea. These results suggest that EGCG may be developed into an effective emergency contraceptive and offer a non-hormonal alternative for women. To establish a model of *in vitro* luteinization, hCG was added to follicles isolated from mouse, rhesus macaque, and human ovaries and culture was continued for 6 days. For all species, estradiol, progesterone, and inhibin levels reflected luteinization post-hCG. These results show that follicular rupture occurs independent of the reticuloendothelial system and ovarian surface epithelium. Moreover, we have developed a rapid *in vitro* assay for ovulation and luteinization, which has identified EGCG as a possible novel contraceptive. Supported by NIH/NIA F30AG040916.

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### Recognition of Naturally Occurring Mutant HCV Peptides by HCV TCR Gene Modified T Cells

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Hepatitis C Virus (HCV) infection is a major public health concern with approximately 3% of the world's population being infected. Liver diseases such as hepatocellular carcinoma (HCC) and cirrhosis are often associated with chronic HCV infection. While both humoral and cellular immunity exist against HCV proteins in HCV-infected individuals, not all patients can mount an effective anti-HCV immune response to clear viral load. Subsequent chronic infections are thought to be attributed to the rapidly mutating HCV genome leading to immune escape variants. Limited effective therapies and slow development of vaccines for HCV infections call for more effective treatments to reduce worldwide morbidity and mortality from HCV infection and HCV-related disease. One immune-based strategy that has shown promise to treat other malignancies such as melanoma is adoptive T cell transfer. This approach uses retroviral vectors encoding T cell receptor (TCR) genes to redirect the specificity of normal peripheral blood lymphocyte (PBL)-derived T cells to recognize tumor-associated antigens. It is believed that such a TCR gene transfer strategy for tumor studies can be extended into the field of HCV biology. However, because HCV mutates its genome to evade the host's immune response, it is important to assess how effective HCV reactive T cells may be against mutant viruses. We have previously identified a novel HCV TCR from an HLA-A2-restricted, HCV NS3:1406-1415-reactive cytotoxic T lymphocyte clone isolated from a patient with resolved HCV infection. We demonstrated that Jurkat 76 cells and PBL-derived T cells transduced with a recombinant retroviral vector encoding the HCV NS3:1406-1415 TCR could recognize peptide-loaded targets and $HCV^+$ HCC cells with cytokine production in a CD8-independent manner. Additionally, it cross-reacted to targets presenting naturally occurring mutant epitopes. We believe that the capability of broad cross-reactivity is not unique to HCV NS3:1406-1415 TCR and can exist in other TCRs found in patients with HCV infections. Here, we showed a recently identified TCR reactive to HCV NS3:1073-1081 to be a high affinity HCV TCR capable of recognizing naturally occurring mutational escape variants in a CD8-independent manner. Data supporting this theory allows for the potential to develop novel TCR-based gene therapy studies for HCV infections and HCV-associated HCC.

# Poster Abstracts

## 190

## Meta-Analysis of Genetic Association Studies Identifies 66 New Loci for Body Mass Index, Confirming a Neuronal Contribution to Body Weight and Implicating Several Novel Pathways

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**Background:** Large-scale genome-wide association studies (GWAS) are elucidating the genetic underpinnings of obesity and related metabolic diseases, which affects more than a third of the U.S. population and may contribute to the development of insulin resistance, dyslipidemia, type 2 diabetes, cardiovascular disease and some forms of cancer. **Methods:** We and others have identified more than 50 loci robustly associated with BMI, WHR, body fat%, and extreme obesity. To identify additional loci that associate with BMI, we expanded the GIANT consortium to include a total of 236,231 individuals from 82 GWAS, as well as 103,046 individuals from 43 studies that were genotyped with the Metabochip, a custom-designed array comprised of SNPs with prior evidence of associations ($P < 1\times10^{-4}$) with metabolic traits. We carried out association analyses in individual studies using an additive genetic model and combined data across groups using a fixed-effects inverse variance meta-analysis approach in METAL. **Results:** We confirmed 31 of the previously established BMI loci and identified 66 new loci associated with BMI ($P < 5\times10^{-8}$). Several of these novel loci are near genes in previously recognized BMI-associated pathways, including neuronal processes (*ELAVL4*, *CREB1*, *GBE1*, *STXBP6*, *GRID1*, *NAV1*) and central appetite regulation (*BBS4*, *ASB4*). Besides, novel loci harboring genes involved in glucose and insulin homeostasis (e.g., *TCF7L2*, *IRS1*, and *GIPR*) and lipid metabolism (e.g., the *APO*-cluster, *NPC1*, *HMGCR*) are now apparent. Conditional analyses across the 97 BMI loci reveal additional secondary associations ($P < 5\times10^{-8}$ for the secondary variant) at five loci (*MC4R*, *BDNF*, *GP2*, *FANCL*, and *ADCY9*). **Conclusions:** These results more than triple the number of identified obesity-susceptibility loci, confirm a neuronal contribution to body weight regulation and implicate additional pathways that further elucidate the possible common genetic etiologies between obesity, glucose, insulin, and lipid homeostasis.

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## Regulation of Synaptic Development by Insulin Signaling in Drosophila

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Synapses, the basic functional units in the nervous system, are the site of pathology in many neurodevelopmental and neurodegenerative conditions, from Autism Spectrum Disorder to Alzheimer's Disease. Understanding the molecular mechanisms required for normal synaptic growth and function is essential for revealing the pathophysiology of synaptic disorders and for developing novel therapies. Using the Drosophila larval neuromuscular junction (NMJ) as a model, I performed a genetic screen and identified the insulin signaling pathway as a novel regulator of synaptic development. This is an especially intriguing result because neuropathy is one of the most common and debilitating sequelae of Diabetes Mellitus. Although a number of mechanisms have been implicated in the progression of diabetic neuropathy, a role for aberrant insulin signaling itself has yet to be identified. My data show that global reduction in insulin signaling results in striking NMJ overgrowth despite undergrowth of most other tissues in the mutant flies. Interestingly, this overgrowth phenotype does not appear to be solely the result of insulin signaling within the cells at the NMJ. First, reduction of insulin signaling specifically in neurons appears to only modestly affect NMJ development. Second, surprisingly, when insulin signaling is reduced either in muscle or glia, I observe NMJ undergrowth, the opposite phenotype of the global mutant. These results suggest that insulin signaling in specific cell types such as glia and muscle leads to positive regulation of NMJ growth. Conversely, in other cell types insulin signaling promotes negative regulation of NMJ growth. Together, my data suggest a role for insulin signaling both in the cells at the NMJ and in other non-neuronal cells to regulate synaptic development. Further experiments will investigate how insulin signaling regulates NMJ growth in these cell types. By elucidating a novel pathway regulating synaptic growth, these experiments will advance our understanding of molecular mechanisms that govern synaptic development and identify novel potential targets for therapeutic intervention for disorders with underlying synaptic pathology.

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## Persistent Activity at Perirhinal and Lateral Entorhinal Cortices Supports Long-Term Trace Conditioning

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Perirhinal (PR) and lateral entorhinal (latEC) cortices, major bidirectional connecting nodes between higher-order cortex and hippocampus, exhibit persistent activity *in vitro* following a brief stimulus. PR is essential for acquisition, but latEC only for retention, of trace eyeblink conditioning (tEBC), suggesting that these parahippocampal areas may possess mechanisms for bridging time between stimuli *in vivo*. We recorded multiple single neurons from PR, latEC and CA1 in the awake rabbit during tEBC, in which a 500ms gap ("trace period") separated conditioned (CS; whisker vibration) and unconditioned stimuli (US; corneal airpuff). Among 408 cells recorded in PR and latEC, 46% were significantly modulated during task performance, showing either an increase or decrease in firing rate during CS presentation or in the trace interval between CS and US. One month after acquisition, rabbits were retrained on tEBC, and 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. We conclude that persistent post-stimulus activity may contribute to binding stimuli together across time, and in latEC, may support long-term (hippocampal-independent) tEBC. Further data are needed to compare roles of PR, latEC and hippocampus pre- and post-consolidation and draw conclusions for network function in long-term memory.

# Poster Abstracts

## 194

### ATG16L1 Deficient Macrophages Enhance Host Defense to Uropathogenic *Escherichia coli* Infection by Increasing Inflammation and Reducing Bacterial Burden

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Proteins of the autophagy pathway, a cellular degradation pathway wherein cytosolic components are targeted for lysosomal degradation, play important roles in pathogen control and modulation of innate immunity. Urinary tract infections (UTIs) are among the most common infectious diseases worldwide and are primarily caused by uropathogenic *Escherichia coli* (UPEC). We recently showed that mice with a deficiency in the autophagy protein, ATG16L1 (Atg16L1$^{HM}$), clear a UTI more rapidly and thoroughly than control mice. We next sought to elucidate the mechanism(s) whereby ATG16L1 regulates and balances pathogen control and innate immunity. Using mice deficient for ATG16L1 in myeloid compartment, we demonstrated that ATG16L1 deficiency in innate immune cells was the primary driver of this clearance and ATG16L1 deficiency was associated with increased recruitment of monocytes to infected bladders upon infection. We next tested the hypothesis that ATG16L1 deficiency in the monocyte/macrophage lineage alters UPEC phagocytosis and inflammatory cytokine secretion. We isolated macrophages from the bone-marrow of Atg16L1$^{HM}$ and control mice and challenged them with UPEC. We found that Atg16L1$^{HM}$ macrophages exhibit a significantly elevated intracellular bacterial load, and produced more IL-1β, a pro-inflammatory cytokine, in response to UPEC challenge than control macrophages, an increase not elicited by avirulent UPEC. Our findings suggest that the loss of ATG16L1 tips the macrophage into a 'hyper-immune' state leading to increased cytokine secretion which may lead to the enhanced immune cell recruitment to the bladder during an infection and thereby result in rapid UPEC clearance in ATG16L1 deficient mice during a UTI. Given that UTIs are common and costly and that antibiotic-resistant pathogens are becoming increasingly prevalent, the potential of this new knowledge to contribute to development of new treatment regimens to elicit an effective immune response to UPEC could be vital.

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### Pre-B Cell Colony-enhancing Factor is Elevated in Patients with Pulmonary Hypertension and Promotes Pulmonary Artery Smooth Muscle Cell Proliferation

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**Introduction:** Pre-B cell colony-enhancing factor (PBEF) is a pro-inflammatory cytokine involved in cell survival, angiogenesis, and NAD biosynthesis, with a putative role in pulmonary vascular remodeling. In this study, we aimed to determine whether PBEF is upregulated in plasma and lung tissue of patients with pulmonary arterial hypertension (PAH) and examined whether PBEF promotes human PASMC (hPASMC) proliferation, a hallmark of PAH. In addition, we investigated the role of PBEF in the regulation of STIM2, an important modulator of store-operated calcium entry (SOCE) and pulmonary vascular remodeling. **Methods**: Plasma PBEF levels were measured in PAH patients (n=91) and controls (n=18) using the Bio-Plex Pro Visfatin immunoassay (Bio-Rad). Whole lung lysates from three PAH patients and three controls were used in western blotting to determine PBEF protein levels. Human PASMCs were plated at 5000 cells/well and stimulated for 48 hrs with PDGF (positive control), PBEF (1, 5, and 20μg/ml doses), or control. Cell proliferation was determined using a colorimetric BrdU Incorporation Assay (Calbiochem). For STIM2 experiments, hPASMCs were stimulated with either PBEF alone (20μg/ml) for 24 hrs, PBEF in combination with FK-866 (PBEF enzymatic inhibitor, 10μM) or α-PBEF antibodies (20μg/ml), or control. STIM2 protein levels were then measured via western blotting. **Results**: PBEF levels were significantly increased in the plasma (PAH patients: median=2623, IQR=1342-6366; Controls: median=1648, IQR=840-2578; p=0.009) and lung tissue ($p<0.05$) of PAH patients versus controls. Stimulation of hPASMCs with PBEF caused a dose-responsive increase in BrdU incorporation (20μg/ml $p<0.001$, 5μg/ml $p<0.001$, 1μg/ml NS). STIM2 protein levels increased with PBEF-stimulation (fold change=2.5, $p<0.04$) and were attenuated by administration of either FK-866 or α-PBEF antibodies. **Conclusion**: These studies demonstrate that PBEF is upregulated in the plasma and lungs of PAH patients. Stimulation of hPASMCs with PBEF resulted in a dose-responsive increase in cell proliferation and increased levels of STIM2 protein, and STIM2 elevation was attenuated using FK-866 or α-PBEF antibodies. These preliminary data suggest that PBEF may be involved in pulmonary vascular remodeling by inducing cell proliferation, possibly via calcium signaling.

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### Salt-sensitive Hypertension Mediated by NCC via OSR1

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**Background:** Recently, it was reported that adrenergic stimulation, a major contributor to salt-sensitive hypertension, activates the thiazide sensitive Na-Cl cotransporter (NCC) in the distal convoluted tubule (DCT). Here, we tested whether NCC activation is required for this effect. **Methods:** Blood pressure (BP) by volume pressure recording tail-cuff. Chronic norepinephrine stimulation: Baseline BP of the mice was measured during week one. Vehicle or norepinephrine (NE, 2.5 mg/kg/d) was administered by osmotic minipump for weeks two and three. Diet was 0.49% NaCl during weeks one and two and 8% NaCl for week three. Acute stimulation: Mice were injected intraperitoneally with NE (750 μg/kg), phenylephrine (PE, 750 μg/kg), isoproterenol (Iso, 750 μg/kg), or vehicle as indicated. Kidneys were harvested 30 minutes later. **Results:** NE infusion increased blood pressure of mice after one week, an effect that was enhanced by dietary salt

loading. Total and phosphorylated kidney NCC (pNCC, a marker of activation) levels were increased after three weeks. The salt-dependent increase in blood pressure was substantially reduced by NCC knockout, confirming that NCC activation is required for the blood pressure effect. To study mechanisms of activation, we performed acute NE administration. Kidneys from mice acutely treated with NE showed increased pNCC (2.83 fold increase, $P<0.01$), while total NCC remained unchanged. To test whether adrenergic effects were mediated by angiotensin II, we examined pNCC in angiotensin II type 1a receptor knockout mice; the effect was unchanged. To determine the adrenergic receptor subtype required, stimulation was performed with PE (alpha adrenergic specific agonist), Iso (beta adrenergic specific agonist), or both. The combination of both increased pNCC more than stimulation by either agonist alone. NE stimulation did not significantly change the abundance or subcellular distribution of STE20/SPS-1-related proline-alanine-rich protein kinase (SPAK), the predominant kinase to phosphorylate NCC. Therefore, we speculated that oxidative stress-related kinase (OSR1), the other kinase known to phosphorylate NCC, might be involved. The increase in pNCC abundance induced by acute NE was preserved in SPAK knockout mice, as detected by both Western blot and immunofluorescence. Further, acute NE increased the apical localization of OSR1 in the DCT of both wild-type and SPAK knockout mice as detected by immunofluorescence staining. **Conclusion:** Norepinephrine activates NCC acutely to cause salt-sensitive hypertension. NCC can be activated independent of SPAK, likely by OSR1. These results identify a novel signaling pathway in the distal nephron.

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### The Binding of *Yersinia* Invasin to β1-integrins Triggers NLRP3 Inflammasome Activation in Gut Epithelial Cells

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The interplay between pathogenic bacteria and the gut epithelium is key in the initiation and progression of gastrointestinal infectious disease. *Yersinia enterocolitica*, a food-borne gastrointestinal pathogen, is highly adapted for invading the intestinal epithelium while simultaneously evading host innate immune responses. The primary immune evasion mechanism of *Yersinia* involves the intoxication of host cells with virulence proteins called *Yersinia* outer proteins (Yops). These Yops disable innate immune defense mechanisms including the production of proinflammatory cytokines. Interleukin-18 is an important cytokine for the clearance of the bacterium and is among the cytokines inhibited by *Yersinia*. The secretion of IL-18 is tightly regulated by a multi-protein complex called the inflammasome, which controls the activation of caspase-1 and subsequent proteolytic maturation of IL-18. Thus far, little is understood about the mechanisms of inflammasome activation in epithelial cells, although the gut epithelium has been identified as an important source of IL-18. In our studies, we have found that the binding of the *Y. enterocolitica* adhesin, invasin, to β1-integrins on the surface of Caco-2 cells, an intestinal epithelial cell line, provides a novel signal for NLRP3 inflammasome activation and IL-18 secretion. Invasin binding to β1-integrins is known to lead to the formation of focal adhesion complexes, which initiate cytoskeletal rearrangements leading to bacterial uptake through the activation of Rho GTPases. Importantly, we identified two *Yersinia* virulence proteins, YopE and YopH, which target Rho GTPases and tyrosine-phosphorylated focal adhesion proteins, respectively, as potent synergistic inhibitors of caspase-1 activation and IL-18 secretion. Overall, our studies have identified a novel mechanism for NLRP3 inflammasome activation in intestinal epithelial cells that is dependent on β1 integrin signaling. Furthermore, our studies revealed a new *Yersinia* immune evasion mechanism utilizing YopE and YopH, which leads to the disruption of IL-18 secretion from intestinal epithelial cells.

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### Nanoparticle Directed Changes in Kidney Inflammation Following Acute Ischemia

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**Study Purpose:** Renal fibrosis is a failure of regenerative mechanisms; strategies aimed towards identifying suitable targets for fibrotic tissue may hold promise in mitigating or preventing progressive fibrosis thereby reducing the need for renal replacement therapy and critical organ shortage. **Methods:** Since type II macrophages are a crucial component of regeneration, our experiments were designed to test nanoparticle delivery of GM-CSF, known to recruit macrophages, and IL-10, known to convert inflammatory macrophages to suppressive, phagocytic type II macrophages capable of clearing debris, initiating matrix deposition, and delivering pro-angiogenic signals. We compared targeting molecules EGF (richly expressed in the kidney) and FSP1 (Fibroblast specific protein 1, known to be expressed following kidney injury) for their ability to deliver either GM-CSF or IL-10 payload after an acute ischemic insult induced by unilateral murine renal pedicle ligation. Since injured vasculature enables nanoparticle delivery to sites of injury, we hypothesized that injured tissue would garner more nanoparticle deposition. Delivery of either IL-10 or GM-CSF nanoparticles or cytokine alone was compared between injured and uninjured kidneys with and without targeting molecules EGF and FSP1. **Results:** With FSP1 targeting, we observed higher IL-10 levels compared to EGF targeting in the injured kidney (190-fold higher) and compared to IV IL-10 alone (206-fold higher). Injured kidneys treated with EGF-targeted GM-CSF nanoparticles had lower levels of GM-CSF (1.29x104 pg/mL/pg total protein) than serum (3.24x104 pg/mL/pg total protein) or in uninjured kidney (1.76x104 pg/mL/pg total protein). **Conclusions:** FSP1 targeting provides superior IL-10 and GM-CSF delivery in the acutely injured kidney. A paradoxical drop of GM-CSF observed in the injured kidney may be due to increased delivery and subsequent endocytosis by infiltrating macrophages found in the acutely injured kidney. These studies support extension of investigation into the functional responses induced by this targeted nanoparticle payload delivery system.

# Oral Presentations

## 201

### Mechanisms of Inflammatory Gene Regulation by Estrogen

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**Background:** In premenopausal women, estrogen (E2) acts as a cardioprotectant, retarding atherosclerosis and mitigating the inflammatory response in vascular tissue after damage. In earlier studies, treatment of ovariectomized mice with E2 following vascular injury resulted in significantly reduced injury compared to mice treated with a control. Our purpose is to determine the molecular mechanisms by which E2 attenuates the inflammatory response to vascular injury by utilizing primary aortic smooth muscle cell (AoSMC) culture. Identification of the molecules that mediate the effects of E2 will potentially aid in the development of drugs that confer the beneficial anti-inflammatory and vasoprotective effects of estrogen. Mouse AoSMC cultures were grown to 95% confluence in 6-well cell culture plates. The cultures were then pretreated with estradiol. To stimulate the inflammatory response, the cells were treated with the cytokine tumor necrosis factor-alpha (TNF-$\alpha$). Transcripts of the genes CCL2, CXCL9, TNF-$\alpha$, and VCAM1 (common inflammatory response genes) were then quantified using reverse transcription and qPCR to measure the suppressive effect of E2. The amount of inflammatory gene transcripts correlated with both the length of time the cells were pretreated with E2 and the presence of certain signaling pathways. Results and Conclusions: Pretreatment with E2 with a minimum time of four hours is required in order for the suppression of the inflammatory response to take place. Mutant cells in which the non-genomic pathway is disrupted failed to suppress inflammatory gene activation in response to TNF-$\alpha$. Knowing the two mechanisms in which estrogen attenuates vascular damage (rapid nongenomic signaling and a slower genomic signaling pathway), the relatively short amount of pretreatment time suggests to us that the estrogen does not directly antagonize TNF-$\alpha$, but rather sets up transcripts to suppress the inflammatory response. Loss of this suppression in the mutants suggests that E2 employs a non-genomic signaling pathway.

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### Dysregulation of the Imprinted DLK1-DIO3 locus in Promyelocytic Leukemia

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Previous small non-coding RNA (sncRNA) transcriptome sequencing has focused on the 15-30 nucleotide (nt) fraction that primarily consists of miRNAs excluding many larger, potentially important sncRNA species. To address this "sequencing gap", we extended our sequencing to include RNAs 15-75nt in length. We report the sncRNA transcriptome sequencing of 34 cases of AML compared to healthy donor bone marrow controls. The most striking example of dysregulation was observed in M3 AML. The *DLK1-DIO3* locus at 14q32.2 contains 41 snoRNA genes belonging to the *SNORD112-114* family in addition to a large cluster of miRNAs. The DLK-DIO3 miRNAs and snoRNAs were massively up-regulated (10-1,000-fold) in M3 AML compared to non-M3 AML cases and healthy donor controls. Analysis of an independent cohort of 187 AML cases (RJW and TJL on behalf of the TCGA AML analysis group) confirmed that dysregulation of sncRNAs in this locus was largely restricted to M3 AML. The *DLK1-DIO3* locus is one of the most characterized imprinted regions in the human genome. The paternally-derived protein coding genes in this locus (*DLK1*, *RTL1* and *DIO3*) showed no dysregulation in contrast to the aberrantly expressed, maternally-derived sncRNAs. Since imprinting at this locus is controlled by methylation of several differentially methylated regions (DMRs), we performed targeted bisulfite sequencing of DMR regions in M3 and non-M3 patient samples; no difference in methylation status of the DMRs was observed. Array based methylation data of the TCGA cohort (n=187) also showed no difference in overall methylation between M3 and non-M3 AML patient samples in the Ch. 14 DLK-DIO3 region. Moreover, based on expressed germline single nucleotide polymorphisms, mono-allelic expression of sncRNAs in this locus was preserved. Together, these data show that imprinting of the *DKL1-DIO3* locus is not disrupted in M3-AML samples, and that dysregulation of the sncRNAs in this region occurs through an imprinting-independent mechanism. In summary, extended sncRNA transcriptome sequencing is a valuable tool which identified massive dysregulation of sncRNAs in the *DKL1-DIO3* locus in M3 AML. The contribution of the highly aberrantly expressed sncRNAs within this locus to leukemogenesis will require additional study and may provide insight into the mechanism behind disease pathogenesis in M3 AML.

# Oral Presentations

## 203

## The Neglected Isoform of Ras: Cellular Expression and Localization of K-Ras4A

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Ras oncogenes are the most frequently mutated in human cancer. The Ras proteins are small GTPases that control critical cellular pathways for growth, proliferation, and survival, which are dysregulated in cancer. The *KRAS* gene encodes two splice variants, K-Ras4A and K-Ras4B. Oncogenic mutations in *KRAS* are found in both isoforms. Despite its importance as the original K-Ras viral oncogene, the vast majority of studies characterizing Ras biology have overlooked the K-Ras4A isoform. However, recent studies have begun to implicate a greater role for K-Ras4A in cancer than previously assumed. Given that K-Ras4A is differentially expressed in human tissues as opposed to the ubiquitously-expressed K-Ras4B isoform, we developed a strategy using real-time PCR to quantitatively measure the amounts of transcript for K-Ras4A and K-Ras4B in human cancer cell lines. We have observed that up to 25% of *KRAS* transcripts in colon cancer cell lines encode K-Ras4A, compared to 10% and fewer in pancreatic and other cancer cell lines. This may explain a difference in phenotype across human tumors with *KRAS* mutations. As K-Ras4A and K-Ras4B differ only in their targeting signals directing membrane association, determining how K-Ras4A is trafficked within the cell is critical in understanding its role distinct from that of K-Ras4B. We investigated how the targeting signals affect K-Ras4A localization. Using live cell imaging studies, we have shown that K-Ras4A is trafficked to the plasma membrane by combined palmitoylation and polybasic interactions in its hypervariable region. Consequently, loss of these interactions affects downstream signaling and results in decreased ERK activation by K-Ras4A from the plasma membrane. Our findings from these studies will provide a better understanding of this elusive isoform and contribute to our knowledge of Ras biology. This work will also have significant implications for the design and development of drugs against cancers driven by K-Ras.

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## Serotype Specific Sialylated Group B *Streptococcus* Capsular Polysaccharide Structure Influences Virulence Functions

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The bacterial pathogen group B *Streptococcus* (GBS) colonizes 20-30% of pregnant women and is a leading cause of neonatal sepsis and meningitis. Newborn GBS infections are commonly differentiated into early onset disease (EOD), which often presents with fulminant pneumonia and sepsis within the first week of life (median onset ~6 h), and late-onset disease (LOD), presenting more indolently with bacteremia and a high incidence of meningitis between one week and several months of age. Among 9 GBS serotypes, each with unique repeating capsular polysaccharide (CPS) structure, all express a terminal a2>3-linked sialic acid residue in a terminal linkage to an underlying galactose. The most common serotypes associated with neonatal infection are type III and type Ia, with type III strains distinctly overrepresented in LOD and meningitis cases. The current study takes advantage of isogenic strains in which heterologous expression of a polymerase gene converts the predominant CPS expression from Ia to III or vice-versa, and seeks to examine whether the unique configuration of the CPS structure influences certain key GBS virulence phenotypes. Compared to a wild-type (WT) serotype Ia strain, induced expression of the serotype III CPS increased adherence and invasion of human brain microvascular endothelial cells (hBMEC). Conversely, a WT serotype III strain had reduced adherence to hBMECs when induced to express the type Ia capsule. Modeling an early step in EOD pathogenesis with human A549 lung epithelial cells, we found the type Ia to III switch was associated with increased adherence and invasion, while the III to Ia switch did not alter these phenotypes. GBS serotype switching in either direction also influenced the specificity of bacterial binding to human Siglec receptors, important immunomodulatory sialic-acid binding lectins expressed on host leukocytes. Finally, WT serotype III GBS strain and its Ia CPS switch variant had similar complement C3b deposition and resistance to neutrophil killing, whereas switching the WT serotype Ia CPS to serotype III led to increased complement C3b deposition and increased susceptibility to neutrophil killing. Together these results indicate that subtle variations in GBS CPS structure can influence key virulence phenotypes including the predilection of serotype III strains for blood-brain barrier invasion.

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## Lymphotoxin Regulates Commensal Responses to Enable Diet-induced Obesity

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The microbiota plays a critical, weight-promoting role in diet-induced obesity (DIO), but the pathways that cause the microbiota to induce weight gain are unknown. It has been argued that the microbiota adapts to variations in diet through a microbiota intrinsic mechanism. We report that mice deficient in lymphotoxin (LT), a key molecule in host immunity, are resistant to DIO. Notably, the feeding behavior of wild-type and *Ltbr*-/- mice is similar and cannot explain differences in weight gain. *Ltbr*-/- mice differ in microbial community composition compared to their heterozygous littermates, including an overgrowth of segmented filamentous bacteria (SFB). Members of the Erysopelotrichi class, previously demonstrated to overgrow in DIO, are correspondingly diminished in *Ltbr*-/- mice. Sequencing of cecal DNA and subsequent metagenomic analysis revealed lower abundance of enzymes involved in complex carbohydrate digestion, which are putative products thought to be a means by which the microbiota adapts to the obese state and contributes to weight gain. Species level changes were also detected, including an overgrowth of SFB in *Ltbr*-/- mice. Furthermore, cecal transplantation from *Ltbr*-/- mice could confer leanness to germ-free recipients. Housing *Ltbr*-/- mice with their obese siblings rescued weight gain,

demonstrating the communicability of the obese phenotype; changes in weight gain correlated with SFB clearance. *Ltbr-/-* animals lacked interleukin 23 (IL-23) and IL-22 that have been previously correlated with SFB. Mice deficient in these pathways also resisted DIO. Restoration of IL-22 was able to rescue weight gain and SFB clearance in *Ltbr-/-* animals, demonstrating that intact mucosal immunity guides diet-induced changes to the microbiota that enable obesity. We conclude that elements of host immunity, which are extrinsic to the microbiota, contribute to changes in the microbiota that induce weight gain in response to altered diet.

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## Patient with Diagnosis of Acute Myocardial Infarction Without Chest Pain: Clinical and Epidemiological Profile

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The typical presentation of acute myocardial infarction (AMI) is characterized by chest pain, tightness in the left side, radiating to the left arm, intensive and prolonged (greater than 20 minutes), which not only improves or has partial relief with rest or sublingual nitrates. The infarction may occur in the absence of pain, but with nausea, malaise, dyspnea, tachycardia or even confusion. **Aim:** To analyze the epidemiology and clinical diagnosis in patients with acute myocardial infarction (AMI) without chest pain. **Methods:** Descriptive study in which data collection was done through interviews with patients or relatives and hospital records. In the period from October 2003 to December 2010, 1005 patients were admitted with a diagnosis of AMI, of which 146 patients were without chest pain. **Results:** Of the 146 patients enrolled, 90 (61.64%) were men. The average age was 67.56 (± 12.49). The most common symptoms were nausea / vomiting (28.77%) and sweating (28.77%). Followed by: epigastric pain (25.34%), dyspnea (23.9%), pallor (21.23%), nonspecific malaise (11.64%), back pain (10.96%), syncope (7,53%), pain in the upper limbs (7.53%), pain in neck and /or jaw (6.85%), irradiation in the left arm (5.48%) and headache (3.43%). This group showed the following risk factors: hypertension, the most prevalent (69.18%), followed by physical inactivity (49.32%), dyslipidemia (41.1%), diabetes (32.93%), stress (33.56%), family history (30.82%), former smoking (30.14%), BMI> 30 (16.44%), smoking (15.07%). Of these patients, 22.6% reported previous angina, 21.92% acute myocardial infarction (AMI), 14.38% CABG (Coronary Artery Bypass Graft Surgery) and 11.64% had stent placed. Regarding the type of AMI, AMI without ST segment was the most prevalent (41.78%), followed by AMI with ST (29.25%) and unstable angina (28.77%). **Conclusions:** AMI without chest pain brings a challenge in an emergency, which does not exclude the diagnosis, because other symptoms and risk factors proved prevalent as older age, hypertension, diabetes, dyslipidemia, hypertension, smoking and the presence of a history of AMI. All these numbers point to the need to establish accurate methods and criteria for rapid identification of those patients at high risk of disease in order to treat them early and appropriately.

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## PXK and Lupus: Defining Novel Immunobiology for an SLE Risk Gene

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Systemic Lupus Erythematous (SLE) is a systemic autoimmune disease with a strong genetic component. Over 50 risk genes have been associated with SLE, many with no immediate biological connection to disease. We previously identified one such gene, PXK, as being a candidate gene associated with SLE in women of European descent. These findings have since been replicated. PXK has additionally been identified as a risk gene for RA as well, suggesting that PXK may have a broad role in the pathobiology of autoimmune disease. In this work we undertake the fine mapping of the PXK genetic locus in an effort to refine the association signal. We identify one independent effect in the region occurring strictly in individuals of European ancestry. In tandem with refinement of the genetic signal, we also attempt to identify the SLE relevant biological import of PXK by examining the role it plays in B cells. PXK has been shown to participate in receptor internalization, and we find using both confocal microscopy and ImageStream technology that PXK colocalizes with the B cell receptor (BCR) upon BCR internalization. These results suggest that PXK may play an important role in the regulation of BCR signaling and B cell differentiation and survival. As B cell regulation is crucial to SLE pathogenesis, understanding the specific changes induced by SLE-associated variants in PXK will provide important insight into SLE pathogenesis.

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## Lrig1 Marks a Basal Subpopulation in the Esophagus Epithelium That Function as Stem Cells

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Homeostasis of the stratified squamous epithelium in the mouse esophagus is maintained by a stem cell population thought to reside in the basal cell layer. Although various candidate stem cells markers have been proposed in the mouse esophagus, none have yet been identified by long term lineage labeling. Lrig1, a negative regulator of the ErbB family of receptor tyrosine kinases, has recently been identified as a marker of largely quiescent, long-lived intestinal and colonic stem cells. We now show that Lrig1 also marks a subset of basal cells in the adult mouse esophagus that appear to function as stem cells. By lineage tracing in $Lrig1^{CreERT2/+}$;$Rosa26R^{LacZ/+}$ mice, we observe that Lrig1-expressing cells are present throughout the entire length of the esophagus. Two days after a single pulse of tamoxifen, labeled cells are initially scattered within the stratified squamous epithelium predominately as single or paired basal cells. With longer labeling (7 to 90 days), $Lrig1^{+}$ cells give rise to suprabasal and superficial squamous cells, as well as additional basal cells. $Lrig1^{+}$ cells persist in all levels of the stratified squamous epithelium at 90 days. $Lrig1^{+}$ basal

cells co-localize with the basal cell marker CK14. Differentiated cells arising from this Lrig1$^+$ basal cell population co-localize with suprabasal and superficial squamous cell markers, respectively, confirming that this cell population can give rise to differentiated lineages. Notably, labeled cells were not observed in the mouse esophagus by short-term and long-term lineage tracing in Lgr5$^{EGFP\text{-}Ires\text{-}CreERT2/+}$;Rosa26R$^{LacZ/+}$ mice. Lrig1$^+$ esophageal epithelial cells are also present at birth and undergo robust expansion and differentiation in early post-natal development, but then appear to remain predominantly in basal clusters at 18 months of age. Studies are currently ongoing to determine the relative frequency and proliferative status of the Lrig1$^+$ basal cell and whether this isolated cell population can support complete epithelial formation in organotypic culture. Nonetheless, these data are the first to demonstrate a squamous epithelial stem cell in the esophagus by lineage tracing and suggest that Lrig1 marks a subset of basal cells involved in esophageal growth and homeostasis. Injury and/or genetic modification of this Lrig1$^+$ basal cell population may provide insight into a cell-of-origin for and critical genetic alterations in Barrett's esophagus and esophageal adenocarcinoma.

## 209

## Src Kinase Regulates the Human Kinesin-5, Eg5, by Phosphorylating Tyrosines in the Eg5 Motor Domain

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**Background**: The human kinesin-5, Eg5 functions as a critical driver of bipolar spindle assembly and maintenance during mitosis. Four Eg5 subunits organize to form an antiparallel dimer of dimers; this arrangement allows the Eg5 tetramer to attach to overlapping MTs in the spindle midzone and push these MTs in opposite directions. Eg5's activity serves to separate the spindle poles during prophase, and contributes to spindle integrity in metaphase. Given this critical mitotic activity, Eg5's mechanism has been the target of many chemotherapeutic drug development efforts. **Results**: Using *in silico*, *in vitro* and cell culture methods, we show that Src kinase phosphorylates specific tyrosine residues in Eg5. These residues are located near the nucleotide pocket and the functionally critical L5 loop. Phosphomimetic and non-phosphorylatable Eg5 mutant proteins have diminished ATPase activity and microtubule sliding relative to wild-type Eg5. We also report that phosphomimetic proteins have greatly reduced affinity for the Eg5 inhibitor S-trityl-L-cysteine. **Conclusions**: These findings suggest that Src phosphorylation of Eg5 may provide cells a non-mutagenesis-dependent strategy to evolve resistance to anti-mitotic Eg5 inhibitors. In this case, combination treatment targeting both Src and Eg5 may inhibit mitosis more effectively than anti-Eg5 treatment alone. Ultimately, Src phosphorylation of Eg5 represents a novel regulatory mechanism for a human kinesin, and links the chemical and physical processes that cause mitosis.

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## IL-4 and IFN-γ Play Opposing Roles in Controlling α-Galcer-induced NKT Cell Hepatitis by Regulating Differentially Neutrophils in Feedback Loops

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A hallmark of NKT cell activation is production of IL-4 and IFN-γ, which mediate many important functions of NKT cells. Injection of mice with the NKT ligand α-Galactosylceramide (α-GalCer) induced mild liver injury and hepatitis with a rapid elevation of IL-4 and a delayed elevation of IFN-γ. Surprisingly, genetic deletion of both cytokines aggravated rather than abolished NKT cell-mediated hepatitis. Furthermore, ablation of IL-4, IL-4R, or its downstream signaling molecule STAT6 reduced α-GalCer-induced neutrophil infiltration, liver injury, and hepatitis. In contrast, deletion of IFN-γ, IFN-γR, or its downstream signaling molecule STAT1 markedly increased neutrophil survival and NKT expansion, thereby exacerbating liver injury and hepatitis. Collectively, our results lead to a model in which activation of NKT cells, on one hand, rapidly releases IL-4, which induces neutrophil recruitment and hepatitis, on the other hand, it sequentially produces IFN-γ, which acts a negative feedback loop to ameliorate α-Galcer-induced NKT cell hepatitis by inducing neutrophil apoptosis and inhibiting NKT cell expansion via a STAT1-dependent manner.

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## Affinity Matured RANKL Identified by Yeast Surface Display Possesses Increased Rank Signaling and Osteoclastogenic Potential

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The interaction between Receptor Activator of NF-κB Ligand (RANKL) and its receptor RANK is essential for the differentiation and function of the osteoclast, the sole bone resorbing cell. Osteoprotegerin (OPG), a soluble homodimer, acts as a decoy receptor for RANKL and thus, inhibits osteoclastogenesis. An imbalance in the RANKL/RANK/OPG axis with decreased OPG and/or increased RANKL is associated with diseases that favor bone loss, including osteoporosis. We have, in the past, used our co-crystal structures of RANKL/RANK and RANKL/OPG to selectively manipulate binding to the signaling receptor RANK. In the present study, we established a yeast surface display system and screened libraries of randomly mutated RANKL proteins to identify mutations that abolish binding to OPG while preserving binding to RANK. After multiple rounds of sorting using equilibrium and kinetic-based approaches, we enriched for RANKL mutants that had lost the ability to recognize OPG while simultaneously retaining binding to RANK. Interestingly, we also identified several RANKL variants possessing substantially higher affinity for RANK as compared to their wild-type counterpart. Using recombinant RANKL mutant proteins, we find that increased affinity RANKL mutants produce more robust signaling

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downstream of RANK and have a greater osteoclastogenic potential. Our results, which are the first to document gain of function RANKL mutations, indicate that the physiological RANKL/RANK interaction is not optimized for maximal signaling and function, perhaps reflecting the need to maintain receptor specificity within the complex TNF superfamily. Additionally, we have identified RANKL mutations conferring a range of affinities for RANK, permitting assessment of the relationship between receptor binding and osteoclastogenic capacity. Our structure-based and yeast surface display-derived insights into manipulating this critical signaling axis may aid in the design of novel anti-resorptive therapies as well as provide an example for the design of other receptor-specific TNF superfamily ligand variants.

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## Functional Role of the R1 Protein in the Lifecycle of Rhesus Monkey Rhadinovirus

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Rhesus monkey rhadinovirus (RRV) serves as an *in vitro* and an *in vivo* model for Kaposi sarcoma-associated herpesvirus (KSHV/HHV-8). At the far left end of the RRV genome is a distinct open-reading frame (ORF) designated R1 whose position is equivalent to that of the saimiri transforming protein (STP) of herpesvirus saimiri (HVS) and the K1 protein of KSHV. Similar to K1, the R1 cytoplasmic tail contains motifs capable of binding to the SH2 domains of protein kinases, and R1 has previously been shown to be capable of activating B lymphocyte signaling. We disrupted the R1 ORF in the RRV genome by inserting a green fluorescence protein (GFP) expression cassette. We compared the replication kinetics of the wild-type virus, a R1-deleted recombinant virus, and a revertant virus by plaque assays and real-time QPCR-based genome quantification assays. We found that deletion of R1 from the RRV genome did not significantly affect viral replication on rhesus fibroblasts. We also examined the ability of the R1-deleted recombinant virus to establish latency in B cells. We found that the R1-deleted recombinant virus was able to establish latency in B cells more efficiently than the wild-type virus and that the R1-deletion mutant underwent less spontaneous reactivation from infected B cells than wild-type RRV.

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## NK Cells in Human Metapneumovirus Infection

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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 mechanisms of protective immunity to this single-stranded RNA virus are not clear. This knowledge will be key in developing therapeutic and preventative strategies. 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. To determine the role of NK cells during the host immune response to HMPV, we infected C57BL/6 mice with HMPV 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 IFNγ and CD107a (a marker for degranulation). Surprisingly, after depleting NK cells in C57BL/6 mice using a monoclonal antibody against NK1.1, a type II transmembrane protein expressed on NK cells, we found that NK-depleted mice actually had greater perivascular and peribronchiolar inflammation found on lung histology as compared to isotype controls. This inflammation, which involved mostly lymphocytic infiltrates, was greatest on day 5 post-infection, and was minimal by day 10. The increase in lung infiltrates correlated with greater airway dysfunction (as measured by breath distension) in NK-depleted mice as compared to control mice. Future experiments will determine how NK-cell depletion affects the adaptive immune response to HMPV infection. The findings from this research will provide insight into the general principles of respiratory paramyoxvirus immunity and pathogenesis, as well as the balance between inflammatory and anti-inflammatory responses to infection in the lung environment.

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## Histone Modification Associated with Prolactin-Induced Transcription

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The peptide hormone prolactin (PRL) promotes normal breast tissue growth and maturation, but PRL also contributes to breast cancer development. PRL signals by binding to the transmembrane PRL receptor (PRLr). The PRLr signals from the cell surface to the nucleus in two ways, 1) by activating canonical signaling pathways and 2) by 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. However, the mechanism by which HMGN2 stimulates transcription 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 to attenuate PRLr signaling. HMGN2 binds to nucleosomes and induces chromatin decompaction.

In particular, HMGN2 can facilitate post-translational histone modifications associated with chromatin decompaction, including acetylation of histone H3 at lysine 14 (H3K14). We hypothesize that HMGN2 causes chromatin decompaction at the 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 chromatin immunoprecipitation (ChIP) for the enrichment of factors involved in chromatin decompaction and transcriptional activation. PRL stimulation was found to increase H3K14 acetylation at the *CISH* promoter in breast cancer cell lines. PRL stimulation also induced the loss of histone H3 at the *CISH* promoter, consistent with chromatin decompaction. Correlating these findings with transcriptional activation, PRL stimulation resulted in increased RNA polymerase II bound at the *CISH* promoter. These data suggest that H3K14 acetylation contributes to PRL-induced transcription, possibly by allowing the transcriptional machinery to better access the promoter DNA. Ongoing studies are examining the role of HMGN2 in facilitating these modifications at PRL-responsive promoters. Funding: NIH F30 CA171858, Malkin Scholar Award.

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## Infection with Soil-transmitted Helminths is Associated with Increased Insulin Sensitivity on Flores Island, Indonesia

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**Background:** The pathogenesis of type-2 diabetes (T2D), involves a disturbance of the energy balance and chronic inflammation. As chronic helminth infection is associated with lower nutritional status and anti-inflammatory response, we hypothesize that helminth infections are associated with increased insulin sensitivity. **Methods:** A cross-sectional study was performed in Flores, Indonesia, an area highly endemic for soil-transmitted helminths. Stool samples from 646 participants aged 18-80 years were collected and screened for *Trichuris trichiura* by microscopy and for *Ascaris lumbricoides*, *Necator americanus, Ancylostoma duodenale, and Strongyloides stercoralis* by qPCR. We collected data on body mass index (BMI), waist-to-hip ratio (WHR), fasting blood glucose (FBG), insulin, high sensitive C-reactive protein level and *E. coli* lipopolysaccharide stimulated cytokines (TNF and IL-10). The homeostatic model assessment for insulin resistance (HOMA-IR) was calculated and the association between helminth infection status and insulin resistance was tested by linear regression adjusted for age, sex and BMI. **Results:** Participants with any helminth infection had lower BMI (kg/m2) (mean difference -0.63, 95%CI [-1.22, 0.02], p=0.044), WHR (-0.01, [-0.02, -0.00], p=0.020) as well as insulin (pmol/L) (0.85, [0.74, 0.98], p=0.023) and HOMA-IR (0.83, [0.73, 0.95], p=0.0075) than uninfected subjects. No association was found between helminth infection and FBG (mmol/L). After adjustment for BMI the association between helminth infection and insulin (mean difference 0.89, 95%CI [0.78, 1.01], p=0.081) as well as HOMA-IR (0.88, [0.77, 0.99], p=0.036) remained. **Conclusions:** Helminths are associated with improved insulin sensitivity which may reflect a decreased degree of systemic inflammation.

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## Adolescents are Insensitive to Punishment-induced Suppression of Cocaine Self-administration

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In humans, adolescence is a period of heightened propensity to develop cocaine addiction. One hallmark of addiction is continuation of drug use despite adverse consequences. We tested the sensitivity of adolescents vs. adults to punishment associated with cocaine. Adolescent and adult rats were trained to self-administer cocaine. Punishment in the form of electric footshock paired with cocaine infusions was administered at various phases of drug use. When footshock was administered upon initial exposure to cocaine, adolescents self-administered more cocaine than adults. When electric footshock was administered after acquisition of cocaine self-administration was established, cocaine intake was suppressed for all ages on the day of punishment. However, the next days, adolescents resumed cocaine taking whereas adults did not. When footshock was administered to aged rats with adolescent- or adult-history of cocaine self-administration, responses to punishment were similar across ages. These results indicate that punishment produces long-term suppression of cocaine taking in adults, but not in adolescents. Such insensitivity of adolescents following punishment may contribute to the high susceptibility of drug addiction during adolescence. Furthermore, these findings have implications for methods used for drug cessation; our data suggests that punishment is not an effective means for drug cessation during adolescence, and prompts to explore other means to more effectively manage adolescent addiction.

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### BTLA: New Biomarker for a Highly Proliferative CD8+ Tumor-infiltrating Lymphocytes (TIL) Subset Associated with Melanoma Regression During Adoptive T-cell Therapy (ACT)

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**Introduction:** Adoptive T-cell therapy (ACT) using tumor-infiltrating lymphocytes (TIL) expanded *ex vivo* together with high-dose IL-2 is a promising approach for the treatment of metastatic melanoma by capitalizing on the ability of the immune system to react against cancer. However, it is not known which phenotypic subsets of T cells in the TIL infusion product are associated with a positive clinical response to ACT. **Methods:** Expanded TIL used to treat 31 metastatic melanoma patients with ACT in a Phase II clinical trial at MD Anderson from 2006 to 2010 were analyzed using flow cytometry with a comprehensive panel of markers distinguishing the main T-cell subsets. These phenotypic markers were then correlated to the type of clinical response based on RECIST criteria. We also isolated different $CD8^+$ T-cell subsets and characterized their functions and global gene expression profiles. **Results:** Overall 15/31 patients (48.4%) responded (PR/CR) to TIL therapy with objective tumor regression. We observed that BTLA expression on $CD8^+$ TIL strongly correlated with positive clinical response ($p = 0.002$). We also found that $CD8^+BTLA^+$ TIL exhibited superior proliferative and survival capacity compared to $CD8^+BTLA^-$ TIL in response to IL-2. In addition, $CD8^+BTLA^+$ TIL produced higher levels of effector cytokines. Using Gene-Set Enrichment Analysis (GSEA), we uncovered a significant enrichment of 14 members of the killer-cell immunoglobulin-like (KIR) receptor family and genes related to T-cell anergy (*Ikfz2, Nr4a3, and Egr2)* in the $CD8^+BTLA^-$ subsets. **Conclusions:** BTLA expression on $CD8^+$ TIL defines a subset of less differentiated, highly proliferative anti-tumor $CD8^+$ T-cells. The expression of a large number of KIR family receptors and anergy-related genes in $CD8^+BTLA^-$ TIL suggest they are terminally differentiated, anergic T cells. Our study provides a molecular basis to explain the positive clinical associations of ACT with more $CD8^+BTLA^+$ TIL, and suggest additional T-cell biomarkers to further characterize responders vs. non-responders to this therapy.

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### Wnt Signaling Mediates Rescue of Intestinal Crypt Cell Defects in Telomerase RNA-deficient Mice with Dysfunctional Telomeres

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Background: Late generation telomerase template RNA-deficient ($mTR^{-/-}$) mice suffer from telomere dysfunction leading to widespread degenerative pathologies, including pronounced defects in the intestine. Intestinal pathologies include inflammation, villus blunting, and high levels of apoptosis in crypt epithelial cells - where intestinal stem cells (ISCs) are located. We have found that bone marrow transplantation (BMT) from EGFP-marked and sex-mismatched wild type (WT) donors dramatically ameliorates the intestinal pathology of mutant recipients. The rescue occurs despite an apparent lack of direct contribution by the donor cells to the mutant epithelium (e.g. via fusion or transdifferentiation). Numerous donor-derived cells of hematopoietic and mesenchymal lineages were apparent in the stroma underlying the mutant epithelium, suggesting the rescue occurring in *trans*. Remarkably, based on TIF assays, this rescue involves improvement in the capped state of telomeres rather than blocking the consequences of uncapped telomeres. We found the expression of Wnt-responsive ISC marker genes (*Ascl2* and*Sox9*) to be diminished in the mutants. Furthermore, mRNA expression profiling indicates that mutant intestinal epithelial cells have broadly reduced expression of components at several levels of the Wnt signaling pathway. Following WT BMT, expression of *Ascl2* and *Sox9* is elevated, suggesting that increased Wnt signaling may mediate the rescue. Supporting this idea, supplementation of the diet with lithium (Li inhibits GSK-3β, akin to Wnt pathway activation), or injection of the Wnt/LRP/LGR5 receptor ligand R-spondin1 (Rspo1), elevates *Ascl2*expression and diminishes apoptosis in the mutants after only seven-to ten days of treatment. We hypothesize the mechanism of rescue is due to a direct improvement of telomere maintenance by upregulation of shelterin proteins, which maintain telomere integrity. Indeed, we find that lithium and 6BIO (another GSK-3β inhibitor) each upregulate the expression of key shelterin proteins Trf2 and Pot1. Our findings indicate that mice with critically shortened telomeres suffer from defects in the ISC niche that can be ameliorated by enhanced Wnt signaling. Furthermore, they indicate that telomere capping can be regulated by extracellular signals.

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## Gender Differences in the Career Outcomes of Johns Hopkins MD-PhD Program Graduates

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Following the rapid expansion of female enrollment in MD-PhD programs in the 1990s, women physician scientists seemed poised to assume leadership roles in government, academia, and industry. Indeed, the mission of the Johns Hopkins MD-PhD Program is to train future leaders in academic medicine who have expertise at the intersection of medicine and fundamental biomedical research. However, decades later, the increase in female MD-PhD students has stalled to less than 35% of matriculants. Female attrition continues to occur at every stage of the "leaky pipeline" to success, from being less likely to pursue research after graduation to facing greater difficulty obtaining promotions. As students of the Association of Women Student MD-PhDs (AWSM) at Johns Hopkins, we have investigated the career outcomes of women from our institution. We compared the current positions and titles of male and female MD-PhD students who graduated from our program between 1980 and 2009. We found that the women graduates are underrepresented in leadership positions in academia, industry, and government; they have less diverse career paths compared to male graduates; and those who remain in academic medicine progress through academic ranks at lower rates than men from the same graduating class. These findings are consistent with a recent national study of physician scientists, which determined that women physician scientists have significantly lower salaries than their male counterparts, independent of rank and number of publications (which were also lower among women). While the outcomes of our graduates are thus likely to be a symptom of gender disparities nationwide and not particular to Johns Hopkins, these findings have galvanized students in the program to strengthen support networks and professional development for the Hopkins MD-PhD Program and AWSM.

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## Novel Mechanisms of S1P Action in Neuroinflammation

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Sterile inflammation is associated with multiple neurological disorders, including Multiple Sclerosis, Alzheimer's disease, and traumatic brain injury, with astrocytes becoming reactive, secreting various cytokines. Interleukin-1, the key molecule of sterile inflammation, subsequently upregulates the expression of numerous cytokines in surrounding cells, including astrocytes. Importantly, IL-1 upregulates the expression of potent chemokines CXCL10 and CCL5, which recruit T cells to sites of sterile inflammation and these chemokines are accordingly elevated in many neuroinflammatory diseases. Neuroinflammation is also characterized by elevated levels of sphingosine-1-phosphate (S1P). Although S1P enhances IL-1-induced expression of many cytokines in astrocytes, it reduces IL-1-induced CXCL10 and CCL5 expression. This inhibition is mediated via S1PR2, which is one of five extracellular G-protein-coupled receptors, and is dependent on $G_{\alpha i}$. However, the detailed mechanism of S1P-mediated inhibition is elusive and is independent of NF-κB and MAPK. We found that expression of CXCL10 and CCL5 is tightly regulated by Interferon Regulatory Factor-1 (IRF-1) with IL-1 robustly inducing the expression IRF-1, nuclear translocation, and binding to the CCL5 and CXCL10 promoters. Surprisingly, S1P does not inhibit these processes. Since IL-1 also activates Rac-1 and PAK-1 in an IRAK dependent mechanism, the effect of PAK and IRAK inhibition was tested. Both IRAK and PAK inhibition blocked IL-1-induced CXCL10 and CCL5 expression, but neither blocked IRF-1 induction, translocation, nor DNA binding. Since IRF-1 activation requires K63 polyubiquitylation and likely phosphorylation, S1P may mediate these post-translation modifications, this hypothesis is currently being investigated.

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## Novel Markers to Identify and Isolate Putative Stem-like Cells in Human Uterine Leiomyoma

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Uterine leiomyoma is the most common benign tumor in reproductive-age women. So far the presence of human uterine leiomyoma stem cells has only been reported by using side population technique (Hoechst 33342 dye exclusion). This study aims to identify cell surface markers for purifying putative stem-like cells in human leiomyoma. Real time PCR and PCR array screening for cell surface markers identified significantly elevated CD49b (Integrin A2 receptor) and CD34 gene expression in leiomyoma side population compared to main population cells. By triple staining with CD34, CD49b, and Hoechst 33342 dye , leiomyoma cells were sorted into three subpopulations including CD34+CD49b+, CD34+CD49b-, and CD34-CD49b- cells, with 95 % of leiomyoma side population cells residing in CD34+CD49b+ cells. Real-time PCR analysis showed that CD34+CD49b+ cells expressed lowest levels of estrogen receptor-alpha and progesterone receptor, while they expressed highest levels of estrogen receptor-beta. The clonogenicity of CD34+CD49b+ subpopulation was markedly greater than CD34+CD49b-, CD34-CD49b-, as well as unsorted cells. Intriguingly, xenografts comprised of CD34+CD49b+ cells grew into relatively larger tumors than those comprised of CD34-CD49b- cells, with CD34+CD49b+ xenografts displaying significantly higher proliferative activity. For the first time our findings identify CD34 and CD49b as novel cell surface markers capable of purifying subpopulation of leiomyoma cells possessing stem cell characteristics, which will provide a novel avenue for human uterine leiomyoma therapy.

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### Aβ1-42 Antibody Producing Plasma Cells in DNA Aβ42 Trimer Immunized Mice Reside Predominantly in the Bone Marrow

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Alzheimer's disease (AD) is the most common form of age-related dementia and affects nearly 40 million people worldwide. Immunotherapy provides a possible avenue for prophylaxis of AD, but a clinical trial (AN1792) in which patients with early AD were immunized with Aβ1-42 peptide was halted after the occurrence of meningoencephalitis in 6% of the immunized people which was attributed to a T cell autoimmune response. DNA vaccination has been shown to have a polarized Th2 immune response that lacks many of the features responsible for inflammation seen in peptide immunizations. In this study, we show a new feature of the DNA Aβ42 trimer elicited B cell immune response and present data for the presence of a long lived plasma cell pool residing within the bone marrow in DNA immunized mice but not in peptide immunized mice. Two groups of mice were analyzed: one group of B6C3F1 mice (n=20) were studied 4 months after the last DNA vaccination, and a second group of Balb/c mice (n=14), which received DNA or peptide immunizations, were analyzed 10 days following the last immunization. The comparison of antibody producing cells in bone marrow and spleen for the DNA and peptide immunized mice with an Antibody Forming Cell (AFC) ELISPOT assay and subsequent ELISAs showed that bone marrow plasma cells from DNA immunized mice produced more anti-Aβ42 IgG producing cells and higher levels of secreted IgG antibodies. In peptide immunized mice, more IgG antibody producing cells were found to reside in the spleen. These data indicate that the bone marrow may be an important reservoir for B cells following DNA Aβ42 immunization and is in line with studies showing that the bone marrow represents an excellent niche for the survival of long lived plasma cells and a lifetime source for antibody producing B cells which are independent of continuous antigen specific stimulation. Further studies are needed to show whether it is possible to define additional phenotypic characteristics for the antigen specific B cell immune response in DNA Aβ42 trimer immunized mice or differences in the Th subsets directly involved in initial signaling events to B cells in the germinal center reactions.

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### Targeting Tryptophan Biosynthesis to Kill Mtb Synergistically with CD4 Immunity

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Most existing and experimental anti-mycobacterial drugs target processes required for bacterial viability *in vitro*. However, during infection, Mtb lives in a complex intracellular environment modulated by diverse immune cell subsets. Thus, Mtb genes involved in immune survival could provide new and tractable drug targets. We sought to identify such candidates by using a transposon mutagenesis screen to determine the Mtb genes specifically required for surviving CD4 T cell-mediated stress. We infected wild type and CD4 T cell deficient mice with a pooled transposon library, and identified 24 genes—including two genes in the tryptophan biosynthesis pathway—that were required only in the presence of CD4 T cells. By using a macrophage-CD4 T cell co-culture system we confirmed that bacterial tryptophan biosynthesis was required to resist bacterial killing. Further, we show that the requirement for bacterial tryptophan biosynthesis stems from the IFN-γ-dependent induction of the tryptophan catabolizing enzyme indoleamine-2,3-dioxygenase (IDO) . In light of this, we hypothesized that blocking endogenous tryptophan biosynthesis would synergistically kill Mtb in the context of infection. We then screened a series of anthranilate analogues for anti-mycobacterial activity, hypothesizing that they might inhibit TrpD, an anthranilate-utilizing enzyme in the tryptophan biosynthesis pathway (6-FABA). Two compounds, 2-amino-5-fluorobenzoic acid (5-FABA) and 2-amino-6-fluorobenzoic acid, had *in vitro* MICs of 1-5 μM and bactericidal activity that was rescued by exogenous tryptophan. Both compounds also killed mycobacteria in macrophages in synergy with IFN-γ. At levels wherein both IFN-γ and 6-FABA inhibit about 50% bacterial growth, the combination therapy of IFN-γ and 6-FABA inhibits 99% of bacterial growth and demonstrates bactericidal activity in macrophages. Targeting virulence factors provides a new paradigm in drug discovery, and here we demonstrate that compounds targeting tryptophan biosynthesis can kill Mtb in synergy with immune activity.

# Poster Abstracts

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## A Novel Role of Estrogen/Kisspeptin in the Development of Inguinal Hernia

***Hong Zhao*[1], Elizabeth K. Pearson[1], Robert T. Chatterton[1], Zhenghong Yu[2], David C. Brooks[1], Takeshi Kurita[1], Diana Berger[3], Francesco J. DeMayo[4], Serdar E. Bulun[1]**

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In the US, more than 1 in 4 men develop symptomatic inguinal hernia, and more than 600,000 inguinal hernia repair surgeries are performed annually. However, the biological or genetic basis of inguinal hernia is currently unknown. Aromatase is a key enzyme for estrogen biosynthesis. Our preliminary data established the causal role of estrogen/estrogen receptor (ER) α in the development of scrotal hernias in our genetically engineered humanized aromatase ($Arom^{hum}$) mouse line containing a single copy of the human aromatase gene. Kisspeptin is the functional mediator of estrogen/ERα in hypothalamus for gonadotropin-releasing hormone secretion. We found highly increased kisspeptin in lower abdominal muscle of $Arom^{hum}$ mice. We will test the hypothesis that local estrogen-dependent increase in kisspeptin expression causes scrotal hernia via estrogen/ERα/kisspeptin-mediated signal transduction in lower abdominal muscle and aromatase inhibitors (AIs) will reverses the development of scrotal hernias through decreased local estrogen production and kisspeptin expression. The causal role of excess estrogen in inguinal hernia will challenge existing paradigms of inguinal hernia etiology. The potential results will change the clinical practice setting for inguinal hernias besides the currently surgery strategies, e.g., use of low-dose AIs for the prevention of inguinal hernia in vulnerable populations such as elderly men.

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## The Role of GATA and FOG Proteins in the Adult Mouse Liver

***R Zheng*[*†], B Rebolledo-Jaramillo[**], RC Hardison[‡], GA Blobel[*†]**

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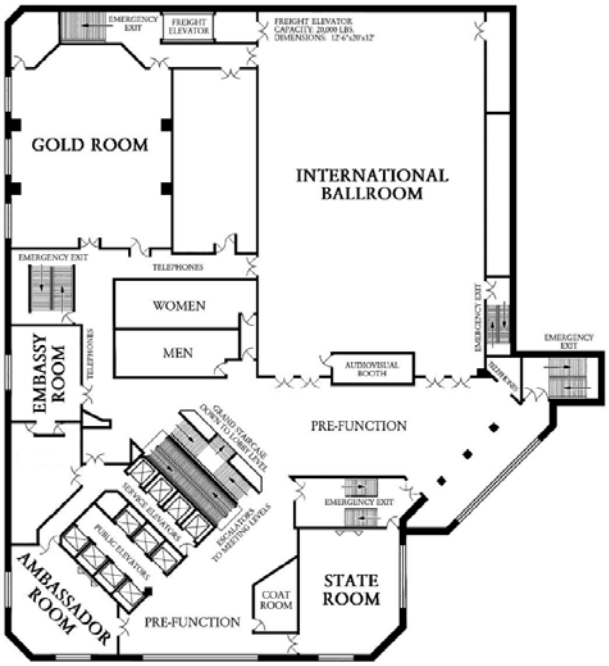
GATA transcription factors and FOG cofactors interact to regulate the development of diverse tissues. Embryonic liver bud outgrowth depends on GATA4 and GATA6, but the function of GATA4, GATA6 and their FOG cofactors in the adult liver remain unclear. FOG1 binds the nucleosome remodeling and deacetylase (NuRD) complex. A subset of aged FOG1 knock-in mice with point mutations in FOG1 that disrupt its interaction with NuRD develop hepatocellular carcinoma. This suggests that FOG1, via NuRD may function as a tumor suppressor. FOG1 functions by associating with GATA factors, thus implicating the liver expressed GATA factors as part of a tumor suppressor pathway. Hence it is essential to understand the normal function of GATA and FOG proteins in the liver. We found that GATA4, GATA6, and FOG1 are the predominant GATA and FOG mRNAs expressed in adult mouse livers. Western blot confirms GATA4 and FOG1 protein expression. To address the discrepancy in previous reports regarding the liver cell type(s) that express GATA factors, we analyzed purified hepatocytes, which express GATA4, GATA6, and FOG1 mRNA. GATA4 and FOG1 proteins are also expressed. Chromatin immunoprecipitation (ChIP) detected GATA4 and FOG1 at liver specific genes, supporting a direct role in the regulation of these genes. To identify liver transcription programs regulated by GATA4, we performed anti-GATA4 ChIP followed by deep sequencing (ChIP-seq) and identified 4409 high confidence peaks. ChIP-qPCR validated our analysis. HOMER motif analysis revealed the consensus sequence WGATAR as the most enriched motif, consistent with GATA4 directly contacting its targets. Genome Region Enrichment of Annotations Tool (GREAT) defined liver metabolism and disease genes among the most enriched gene ontologies. To study GATA4 function in the adult liver, we conditionally deleted GATA4 in hepatocytes, with GATA4 excision confirmed by mRNA and protein expression and ChIP. Studies of the effects of GATA4 depletion on liver morphology and hepatocyte transcriptome will be discussed at the conference. In summary, we provide for the first time a broad analysis of GATA and FOG protein expression and function in the adult liver.

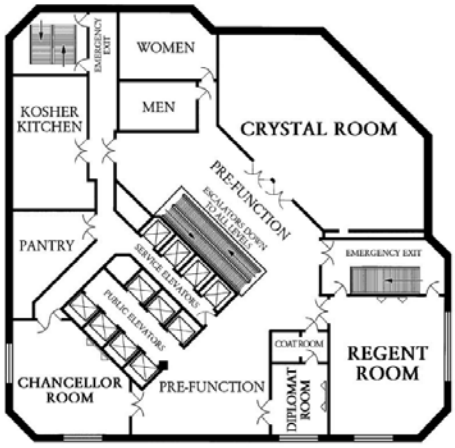
## Notes

# Hotel Floor Plans

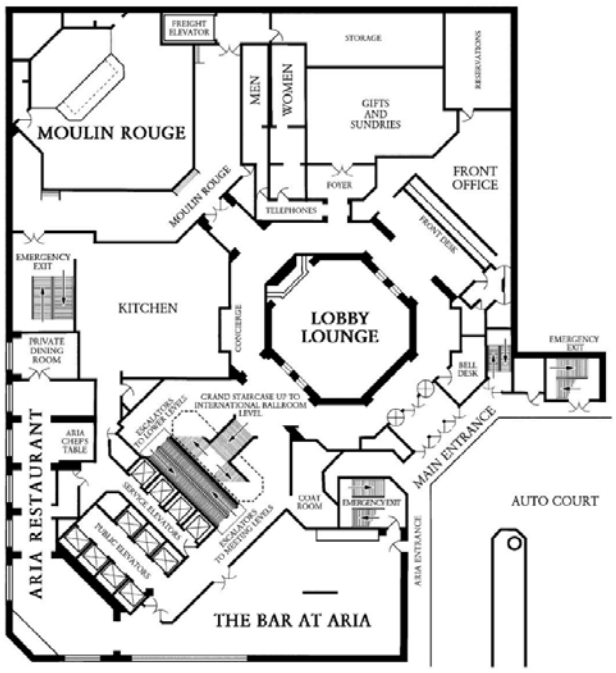
## International Ballroom Level (2nd level)



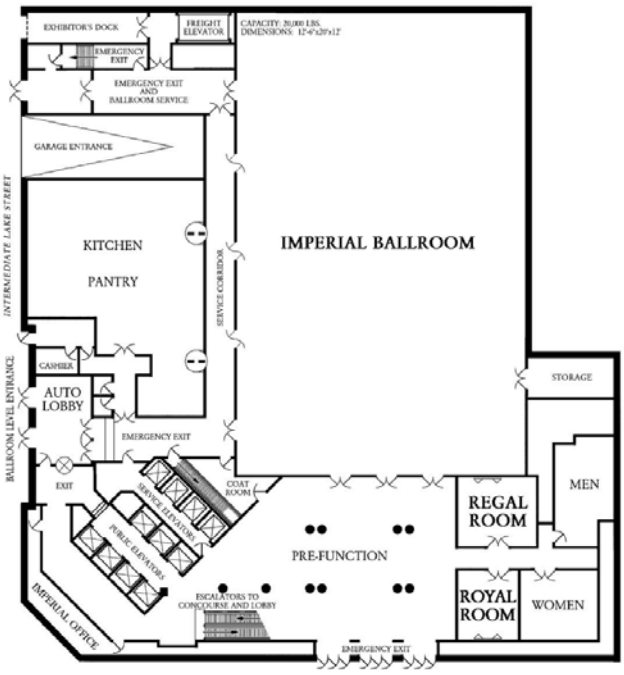

## Meeting Room Level (3rd level)




## Lobby Level (1st level)




## Imperial Ballroom Level (B2)



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