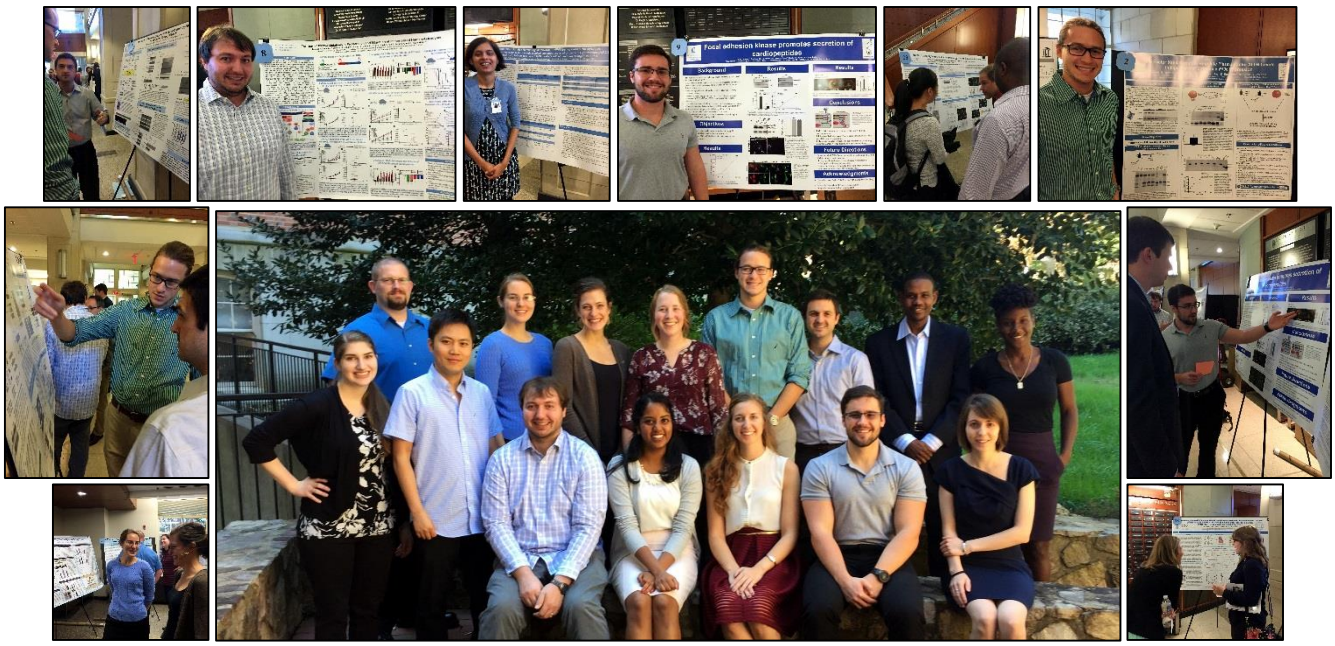
University of North Carolina School of Medicine
Department of Pathology and Laboratory Medicine

Annual Research Symposium

September 7, 2017



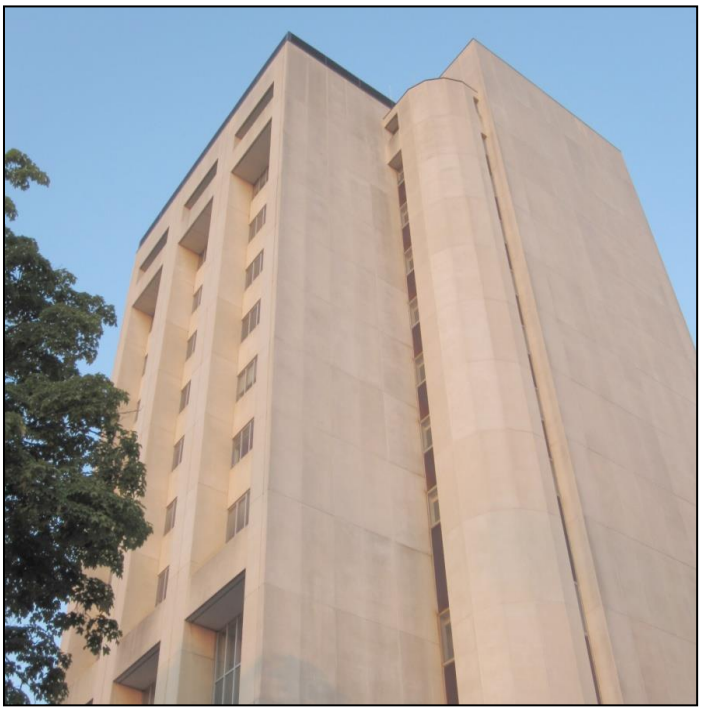
Pathobiology and Translational Science

Department of Pathology and Laboratory Medicine

Annual Research Symposium

Highlighting the research conducted by our Predoctoral Students, and Postdoctoral/Clinical Fellows

September 7, 2017



The Brinkhous-Bullitt Building

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In Memory and Remembrance of

Oliver Smithies, D.Phil.

Kay M. and Van L. Weatherspoon Eminent Distinguished Professor of Pathology and Laboratory Medicine

The 2017 Annual Research Symposium of the Department of Pathology and Laboratory Medicine is dedicated to the memory of our cherished friend and colleague Dr. Oliver Smithies who passed away on January 10, 2017 at the age of 91. Dr. Smithies earned a B.A. with First Class Honors in Physiology at Balliol College of Oxford University in 1946, and then earned a D.Phil. in biochemistry from Oxford University in 1951. Upon completing his doctorate, Dr. Smithies performed postdoctoral research from 1951-1953 in physical chemistry at the University of Wisconsin at Madison. After completing his postdoctoral research, Dr. Smithies served as a Research Assistant and then a Research Associate in the Connaught Medical Research Laboratory at the University of Toronto (1953-1960). In 1960, Dr. Smithies returned to the University of Wisconsin at Madison where he moved through the faculty ranks ultimately becoming the Hilldale Professor of Genetics and Medical Genetics. Dr. Smithies remained in Madison for 28 years before joining the faculty of the Department of Pathology and Laboratory Medicine at the University of North Carolina in 1988, where he continued to work as a bench scientist until his death. Most recently, Dr. Smithies served as the Kay M. and Van L. Weatherspoon Eminent Distinguished Professor of Pathology and Laboratory Medicine.

Dr. Smithies had a long and exceptionally productive career. He published his first paper in 1948 (Ogston and Smithies: Some thermodynamic and kinetic aspects of metabolic phosphorylation. *Physiol. Revs*. **28**:283-303, 1948), and >350 papers in total. Dr. Smithies' research was not only prolific it was also impactful. During the mid-1950s, Dr. Smithies developed a method for starch gel electrophoresis that became the precursor for other electrophoretic methods that combine molecular sieving with protein separation by charge (Smithies: Grouped variations in the occurrence of new protein components in normal human serum. *Nature* **175**:307, 1955; Smithies: Zone electrophoresis in starch gels - Group variations in the serum proteins of normal human adults. *Biochem. J.* **61**:629-641, 1955). In recognition of the importance of this work, the *American Society of Human Genetics* recognized Dr. Smithies with the 1964 William Allen Memorial Award for "…*outstanding work in human genetics, in recognition of development of starch gel electrophoresis, and of important work on the heredity of the haptoglobins, transferrins, and gamma globulins*..." This work was also recognized in 1984 when the *American Association of Blood Banks* recognized Dr. Smithies with the Karl Landsteiner Memorial Award for "…*the development of zone electrophoresis using starch gels, the discovery of the genetic polymorphism of haptoglobin and the insight provided on the role of chromosomal rearrangement and gene duplication in the evolution of protein structure*…," and again in 1990 when Dr. Smithies received the Gairdner Foundation International Award for "…*the discovery, development and application of gel electrophoresis methods that allow the separation and identification of specific proteins and nucleic acids*…." Dr. Smithies continued to follow his interests into new projects that provided opportunities for new technical innovations. During the 1980s, Dr. Smithies work focused on homologous recombination of DNA, which led to the development of new

methods of gene targeting (Smithies *et al.*: Insertion of DNA sequences into the human chromosomal β globin locus via homologous recombination. *Nature* **317**:230-234, 1985). Technical developments related to gene targeting from Dr. Smithies and others formed the foundation for the development of genetically-modified mouse models (transgenic, knockout, and knock-in mice). In 1993, Dr. Smithies received a second Gairdner Foundation International Award for "…*pioneering work in the use of homologous recombination to generate targeted mutations in the mouse*…" This was the first of many awards related to his work in homologous recombination. In 1996, Dr. Smithies received the Ciba Award for Hypertension Research for "…*his groundbreaking work in the use of homologous recombination to insert altered genes into specified positions in the DNA of living cells and the application of this technique to transfer 'designer mutations' to living animals and to the study of high blood pressure and cardiovascular diseases*…" In 1998, Dr. Smithies was recognized as a Foreign Member of the Royal Society of London for "…*his contributions to advancing the knowledge of recombination events in humans, and for applying this knowledge to innovate gene targeting in mammalian cells*…" and received the *Association of American Medical Colleges* Award for Distinguished Research in the Biomedical Sciences for "…*the landmark work that has made possible the only technology for directed mutagenesis in mammals*…" as well as the Research Achievement Award from the *American Heart Association* for "…*his extraordinary scientific accomplishments including innovative approaches in the modification of genes that have expanded the horizons of cardiovascular science and opened the door to improved treatments for heart and blood vessel diseases*..." In 2001, Dr. Smithies shared the Albert Lasker Basic Medical Research Award with Dr. Mario R. Capecchi and Sir Martin J. Evans, for "…*the development of a powerful technology for manipulating the mouse genome with exquisite precision, which allows the creation of animal models of human disease*..." In 2007, Dr. Smithies shared the Nobel Prize for Physiology or Medicine with Dr. Mario R. Capecchi and Sir Martin J. Evans, for "…*their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells...*" These represent just some of Dr. Smithies many awards recognizing the impact of his research on various aspects of biomedical science. In addition to these awards, Dr. Smithies was elected to the National Academy of Sciences in 1971, the American Academy of Arts and Sciences in 1978, the Institute of Medicine in 2003, the American Association for Cancer Research Academy in 2013, and as a Charter Fellow in the National Academy of Inventors in 2013. Dr. Smithies delivered honorary lectures at numerous universities, and received honorary degrees from several institutions. Among these are an Honorary Doctorate of Science from the University of Toronto, an Honorary Doctor of Science from the University of Wisconsin at Madison in 2009, and an Honorary Doctor of Science from Oxford University in 2011.

Dr. Smithies' scientific career and accomplishments are truly impressive and quite exceptional. However, for those of us who had the privilege to know him and work with him, the papers, awards, and honors do not reflect his most important attributes. Despite his tremendous accomplishments, status in the field, and numerous awards and honors, Dr. Smithies is most often described as unpretentious and approachable, and is renowned as a person of gentle character, generous spirit, infectious curiosity, and possessing an enormous passion for science. He served as a formal research mentor for numerous young scientists over the years, and was considered a mentor by many others (including his peers). Dr. Smithies was a cherished colleague to everyone at the University of North Carolina. He was a friend to all and was eager to help others succeed (which he did time after time). Dr. Beverly H. Koller (Departments of Medicine and Genetics, University of North Carolina School of Medicine) was a postdoctoral fellow in Dr. Smithies' laboratory at the University of Wisconsin at Madison, and worked with Dr. Smithies for many years. Dr. Koller reflected on her memories of Dr. Smithies in an obituary published earlier this year [Koller: Oliver Smithies (1925-2017). *Cell* **168**:743-744, 2017]. The following excerpt from Dr. Koller's obituary aptly describes Dr. Smithies as most of us saw him: "…***When celebrating Oliver's incredible life, I believe that we mourn not only the loss of a friend and mentor, but also of an ideal. In our hearts, we know that what Oliver represented was larger than a single life.*** **[He]** ***represented the freedom to be an explorer, to enjoy a lifetime of discovery, to continually ask why, and to continually challenge the consensus***…"

We are proud to have known Dr. Oliver Smithies for many years and we are grateful for the exceptional example he provided for us as a distinguished and accomplished experimental pathologist, and a genuinely good person. Even though he is gone, Dr. Smithies will continue to inspire the generations of scientists who were fortunate enough to have known him to take chances and to do their best work. The 2017 Annual Research Symposium is dedicated to his memory and the scientific ideal that he represented.

Thursday, September 7, 2017 - G100 Bondurant Hall

Schedule of Events

8:55 **Introduction and Welcoming Remarks**
J. Charles Jennette, M.D., Brinkhous Distinguished Professor of Pathology and Chair

9:00-11:00 **Oral Presentations by Graduate Students and Postdoctoral Fellows**
Moderator: Sabri Abdelwahab

Identifying Drivers of SCCOHT Tumorigenesis Following BRG1 Loss by Integrative ATAC/RNA-seq
Krystal A. Orlando, Jessica D. Lang, William P.D. Hendricks, Jeffrey M. Trent, Bernard E. Weissman

Plasma-, But Not Platelet-factor XIII Promotes Red Blood Cell Retention in Contracted Clots and Mediates Clot Size During Venous Thrombosis
Sravya Kattula, James R. Byrnes, Sara M. Martin, Brian C. Cooley, Matthew J. Flick, Alisa S. Wolberg

Detecting Autoreactive CD4+ T Cells and Pathogenic Epitopes in Patients with MPO-ANCA Vasculitis
Katherine G. Stember, Jacob J. Hess, Candace D. Henderson, Susan L. Hogan, Yichun Hu, Simon A. Mallal, Bjoern Peters, J. Charles Jennette, Ronald J. Falk, Dominic J. Ciavatta, Meghan E. Free

Type II Inflammation and Airway Mucosal Dynamics
Bethany Batson, Giorgia Radicioni, Jerome Carpenter, Yuanli Li, Mehmet Kesimer

A Population of Oral Epithelial Label-retaining Cells Resides in a Discrete Niche in Palatal Rugae Ridges
Kevin M. Byrd, Jeet Patel, Scott Williams

Non-targeted Metabolomics Identifies Exercise-induced Cardioprotective Metabolic Pathways That Negate Ischemia Reperfusion Injury
Traci L. Parry, Joseph W. Starnes, Amro Ilaiwy, James R. Bain, Mike J. Muehlbauer, Aubree Honcoop, Christopher B. Newgard, Peter Christopher, Monte S. Willis

11:00-11:15 **Break and Refreshments**

11:15-12:30 **Keynote Presentation**
Moderator: Rachel Dee

Gene Dosage Experiments: How Mouse Genetics Can Model Human Disease
Kathleen Caron, Ph.D., Professor and Chair, Department of Cell Biology and Physiology, UNC McAllister Heart Institute, University of North Carolina School of Medicine

12:30-2:00 **Poster Sessions, Lunch, and Refreshments**
12:00-1:15 Presentation of odd-numbered posters
1:15-2:00 Presentation of even-numbered posters

Graduate Student, Postdoctoral Fellow, and Clinical Fellow Speaker Abstracts

Identifying Drivers of SCCOHT Tumorigenesis Following BRG1 Loss by Integrative ATAC/RNA-seq

Krystal A. Orlando[1], Jessica D. Lang[2], William P.D. Hendricks[2], Jeffrey M. Trent[2], and Bernard E. Weissman[1,3]

[1]Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, NC; [2]Division of Integrated Cancer Genomics, Translational Genomics Research Institute (TGen), Phoenix, AZ; [3]Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC

Over 94% of small cell carcinomas of the ovary, hypercalcemic type (SCCOHT), a rare and aggressive form of ovarian cancer, have mutations and concomitant protein loss in *SMARCA4 (BRG1)*, one of the two mutually exclusive ATPases of the SWI/SNF chromatin remodeling complex. SCCOHT tumors rarely have secondary mutations, making them an excellent model for understanding the role BRG1 and SWI/SNF complexes play in tumor suppression. We *hypothesize that BRG1 loss drives SCCOHT tumorigenesis by altering chromatin accessibility and gene expression.* We have previously shown that BRG1 re-expression in SCCOHT cell lines suppresses cell growth and induces neuronal-like morphology. To identify the top genes and transcription factors driving SCCOHT tumorigenesis, we performed ATAC-seq and RNA-seq in a SCCOHT cell line +/- BRG1 re-expression. BRG1 re-expression increased overall chromatin accessibly, shown by an increase in the number of ATAC-seq peaks, as well as an enrichment in peaks possessing transcription factor binding motifs from FOS/JUN/AP-1, TEAD, and SOX family members. RNA-seq analysis demonstrated that BRG1 re-expression upregulated more genes overall, consistent with the increase in ATAC-seq peaks. Preliminary pathway analysis identified enrichments in epithelial-mesenchymal transition, extracellular matrix remodeling, and KRAS signaling. Future studies include integration of the ATAC/RNA-seq data to further identify correlations between gene expression changes and enriched transcription factor binding motifs and ChIP-seq analysis of SWI/SNF complex members. These studies will uncover the key genes and transcription factors affected by BRG1 loss, provide insight into BRG1's role in SCCOHT tumorigenesis, and potentially yield novel therapeutic targets.

Plasma-, But Not Platelet-factor XIII Promotes Red Blood Cell Retention in Contracted Clots and Mediates Clot Size During Venous Thrombosis

Sravya Kattula[1], James R. Byrnes[1], Sara M. Martin[1], Brian C. Cooley[1], Matthew J. Flick[2], and Alisa S. Wolberg[1]

[1]Department of Pathology and Laboratory Medicine and McAllister Heart Institute, University of North Carolina at Chapel Hill, Chapel Hill, NC. [2]Division of Experimental Hematology and Cancer Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH

Background: The transglutaminase factor XIII (FXIII) plays a seminal role in venous thrombosis by crosslinking fibrin α-chains and promoting red blood cell (RBC) retention in contracted clots. However, the contributions of plasma ($FXIII_{plasma}$)- vs. platelet ($FXIII_{plt}$)-derived FXIII to clot composition, thrombosis, and hemostasis remain undefined. **Aims:** Determine the role of $FXIII_{plasma}$ and $FXIII_{plt}$ in clot contraction, composition, and size. Identify the level of FXIII deficiency that reduces thrombus weight without increasing bleeding. **Methods:** Thrombin generation, FXIII activation, and fibrin crosslinking were measured in whole blood, platelet-rich plasma (PRP) and reconstituted whole blood and PRP from $F13a^{+/+}$, $F13a^{+/-}$, and $F13a^{-/-}$ mice by calibrated automated thrombography and western blotting. Mice were subjected

to inferior vena cava thrombosis, as well as tail transection and saphenous vein bleeding assays. **Results:** FXIII activation and fibrin crosslinking were delayed in *F13a*$^{+/-}$ PRP relative to *F13a*$^{+/+}$ PRP, but thrombin generation and clot contraction were similar in PRP across genotypes. In reconstituted assays, PRP containing $FXIII_{plasma}$, but not $FXIII_{plt}$, formed fibrin α-chain crosslinks. Similarly, the absence of $FXIII_{plasma}$, but not $FXIII_{plt}$, decreased RBC retention in reconstituted whole blood contracted clots, resulting in smaller clots. In vitro and in vivo, FXIII deficiency reduced thrombus weight in a gene dose-dependent manner. In hemostatic challenges, *F13a*$^{-/-}$, but not *F13a*$^{+/-}$, mice had prolonged tail bleeding times, whereas no genotype-dependent difference was observed following saphenous vein puncture. **Conclusions:** FXIII deficiency results in a gene dose-dependent decrease in thrombus weight without altering thrombin generation or platelet contraction. $FXIII_{plasma}$, but not $FXIII_{plt}$, mediates fibrin crosslinking, promoting RBC retention and increased thrombus weight. Imposition of mild-to-moderate FXIII deficiency may reduce venous thrombosis without simultaneously increasing bleeding risk.

Detecting Autoreactive CD4+ T Cells and Pathogenic Epitopes in Patients with MPO-ANCA Vasculitis

Katherine G. Stember[1], Jacob J. Hess[2], Candace D. Henderson[2], Susan L. Hogan[2], Yichun Hu[2], Simon A. Mallal[3], Bjoern Peters[4], J. Charles Jennette[1], Ronald J. Falk[5], Dominic J. Ciavatta[6], and Meghan E. Free[2]

[1]UNC Dept. of Pathology and Laboratory Medicine, [2] UNC Kidney Center, [3]Vanderbilt Center for Translational and Clinical Immunology, [4]La Jolla Institute for Allergy and Immunology,[5]UNC Dept. of Medicine, [6]UNC Dept. of Genetics and Molecular Biology

Objective: To determine if patients with myeloperoxidase specific anti-neutrophil cytoplasmic autoantibody (MPO-ANCA) vasculitis have CD4+ T cells that recognize previously identified MPO epitopes. Background: Several GWAS studies found an association between ANCA vasculitis and human leukocyte antigen (HLA). MHC II receptors (encoded by HLA genes) are key in presenting peptides to the immune system for cellular activation. Previous studies in both mouse and human have identified potential pathogenic MPO epitopes. **Methods:** HLA sequencing was performed on a cohort of 107 patients with MPO-ANCA vasculitis. Sequencing results were used to create MHC II tetramers loaded with MPO epitopes predicted using *in silico* studies, in addition to a scrambled epitope control for both DPB1*04:01 and DRB4*01:01. Patient PBMCs were incubated with tetramers, stained with surface markers, and analyzed by flow cytometry day 1 *ex vivo*. **Results:** Patients carrying DPB1*04:01 and DRB4*01:01 demonstrated specific CD4+ T cell recognition of tetramers carrying disease relevant MPO epitopes. The majority of tetramer positive cells are $CD25^{intermediate}$ memory cells, positive for CD45RO and CCR7. Additionally, tetramer positive CD4+ T cells secrete IL-17 when stimulated. **Conclusions:** Patient CD4+ T cells show significant reactivity to tetramers carrying the human homolog of an epitope described as immunodominant in a mouse model of anti-MPO glomerulonephritis (GN). Recently, it was shown that this same epitope could be used to induce tolerance and attenuate disease in a mouse model of anti-MPO GN. Ideally, we will use this region of MPO to inform the development of new targeted therapies for patients with ANCA vasculitis.

Type II Inflammation and Airway Mucosal Dynamics

Bethany Batson, Giorgia Radicioni, Jerome Carpenter, Yuanli Li, and Mehmet Kesimer

Department of Pathology and Lab Medicine, Marsico Lung Institute, University of North Carolina, Chapel Hill, Chapel Hill, NC

Objective: Airway remodeling including alterations in respiratory mucus properties can lead to airway obstruction, a characteristic of asthma which greatly contributes to morbidity. Our recent work using an *in vitro* type II inflammation model revealed drastic changes in secretion patterns of both mucins and mucin

interacting proteins. We hypothesize that these changes lead to the development of the adherent static mucus plugs characteristic of asthma and therefore are important factors in asthma pathogenesis. **Methods**: Apical and basal secretions from IL-13 challenged primary human tracheobronchial epithelial cell cultures were collected for 20 days and subjected to mucin/mucus measurements for biochemical/biophysical and proteomics characterization. A quantitative proteomic comparison of whole secretions and mucin rich fractions, was made to identify mucin interacting proteins. These proteins were then purified to determine the nature of their interaction with mucin and how this affects the rheological properties of mucus. **Results:** IL13 treatment increased MUC5AC secretion though MUC5B initially decreased. Through immunofluorescent confocal analysis both mucins appeared adherent to the cell surface. Additionally, the secreted mucins had altered macromolecular properties as indicated by SEC-MALLS. Proteomic analysis revealed several potential mucin-interacting proteins that increase in response to IL-13, including FCGBP, which was further overexpressed and studies are ongoing to determine its structure, how it interacts with mucin, and its function in health and disease. **Conclusion:** Our observations indicate that type II inflammation affects mucin macromeric structure, and the secretion patterns/rates of airway mucins and their interacting proteins resulting in aberrant mucus properties, thus contributing to asthma pathogenesis.

A Population of Oral Epithelial Label-retaining Cells Resides in a Discrete Niche in Palatal Rugae Ridges

Kevin M. Byrd, Jeet Patel, and Scott Williams

Department of Pathology and Laboratory Medicine, University of North Carolina School of Medicine, Chapel Hill, NC

The oral epithelia are a collection of stratified epithelia that exhibit rapid turnover and a remarkable capacity for regeneration; however, little is known about their development, maintenance, and renewal. Furthermore, oral stem cells remain poorly characterized. In tissues such as the intestine, both "active" and slow-cycling "reserve" stem cell pools exist which play roles in maintenance and repair during homeostasis and wounding. Here, we adopt an unbiased genetic label retention approach to identify slow-cycling, label-retaining cells (LRCs) in the oral cavity. We have used two distinct promoters (Krt14 and Krt5) to drive expression of a doxycycline-regulatable histone H2B-GFP, which is diluted as cells divide during a variable chase period. We identified discrete pockets of GFP-hi LRCs in regions of the oral cavity, including the oropharynx, salivary glands and palate. Notably, palatal LRCs retain GFP expression for 4 weeks or more, and are associated with rugae ridges. We isolated GFP-hi LRCs by FACS and compared their gene expression profiles to GFP-lo non-LRCs. Palatal LRCs are enriched for the transcription factor Sox9, as well as the TA isoform of p63, while the ΔN p63 isoform is enriched in the GFP-lo population. Lineage tracing studies using K14-CreERT2 and Sox9-CreERT2 have revealed clonal populations with distinct sizes and morphologies reminiscent of stem and transit-amplifying cells. Finally, we show GFP-hi and GFP-lo populations displays different division orientation patterns, with the former characterized by more planar/symmetric divisions. Collectively, these data suggest palatal epithelia harbor stem cell niches that maintain reserve stem cell populations. Funding: This work has been supported by the Sidney Kimmel Foundation (SKF-165 to S.E.W.), NIH/NIDCR (K08DE026537 to K.B.), and a CGIBD Pilot/Feasibility grant (supported by P30 DK034987 to S.E.W.)

Non-targeted Metabolomics Identifies Exercise-induced Cardioprotective Metabolic Pathways That Negate Ischemia Reperfusion Injury

Traci L. Parry[1], Joseph W. Starnes[2], Amro Ilaiwy[1], James R. Bain[3], Mike J. Muehlbauer[3], Aubree Honcoop[1], Christopher B. Newgard[3], Peter Christopher[2], and Monte S. Willis[1]

[1]University of North Carolina, Chapel Hill, NC. [2]University of North Carolina, Greensboro, NC. [3]Duke University, Durham, NC

The effects of exercise on the heart and its resistance to disease are well-documented. Recent studies have identified exercise-induced resistance to arrhythmia is due to the preservation of mitochondrial membrane potential. To identify novel metabolic changes that occurr parallel to these mitochondrial alterations, we performed non-targeted metabolomics analysis on hearts from sedentary and exercise-trained rats challenged with isolated heart ischemia-reperfusion injury (I/R). Exercise training consisted of a progressive treadmill training protocol for five days/week for six weeks. The recovery of pre-ischemic function for sedentary rat hearts was 28.8±5.4% (N=12) compared to exercise trained hearts which recovered 51.9%±5.7 (N=14, $p<0.001$). Non-targeted GC-MS metabolomics analysis of 1) Sedentary rat hearts; 2) Exercise-trained rat hearts; 3) Sedentary rat hearts challenged with global ischemia-reperfusion (I/R) injury; and 4) Exercise-trained rat hearts challenged with global I/R (10/group) revealed 20 significantly different metabolites ($p<0.001$). Enrichment analysis of these metabolites for pathway-associated metabolic sets indicated a >10 fold enrichment for ammonia recycling and protein biosynthesis (L-Glutamic acid; L-Proline; L-Histidine; L-Serine; L-Aspartic acid; L-Glutamine; p<=4.05E-05, FDR=0.0024). Subsequent comparison of the sedentary hearts post-I/R and exercise-trained hearts post-I/R further identified significant differences in metabolites related to Aminoacyl-tRNA biosynthesis and nitrogen metabolism (p<=1.24E-05, FDR<=5.07E-4). CONCLUSION: These studies shed light on novel mechanisms in which exercise-induced cardioprotection occurs in I/R which complement both the mitochondrial stabilization and antioxidant mechanisms previously described. These findings also link protein synthesis and protein degradation (protein quality control mechanisms) with exercise-induced cardioprotection and mitochondrial susceptibility for the first time in cardiac I/R.

Keynote Lecturer

Kathleen M. Caron, Ph.D.
Professor and Chair
Department of Cell Biology and Physiology

Dr. Kathleen M. Caron is a Professor and Chair in the Department of Cell Biology and Physiology in the School of Medicine at the University of North Carolina at Chapel Hill. Prior to her role as Department Chair, Dr. Caron served as Assistant Dean for Research in the UNC School of Medicine. Dr. Caron graduated from Emory University with a B.S. in Biology and a B.A. in Philosophy. For her graduate work, she trained with Dr. Keith L. Parker in the Department of Cell Biology at Duke University where she elucidated the role of steroidogenesis in regulating sexual determination and adrenal and gonadal development using genetic mouse models. To gain more experience in gene targeting approaches, Dr. Caron pursued her postdoctoral training in the laboratory of Nobel Laureate Dr. Oliver Smithies at the University of North Carolina, where she was the first to discover the essential role of adrenomedullin peptide for embryonic survival. Dr. Caron directs a well-funded and highly productive laboratory that uses sophisticated gene targeting approaches to model human disease in mice. With a special emphasis on vascular biology, the Caron laboratory has gained valuable insights into the genetic basis and pathophysiology of lymphatic vascular disease, preeclampsia and sex-dependent cardiovascular disease. Dr. Caron has published over 70 papers and book chapters, and has received numerous awards including a Burroughs Wellcome Fund Career Award in the Biomedical Sciences, an Established Investigator Award and an Innovator Award from the American Heart Association and a Jefferson Pilot Award in Biomedical Sciences. She currently serves as an Associate Editor for the Journal of Clinical Investigation, is a member of several other editorial boards, and holds numerous scientific advisory roles in academia, industry and the National Institutes of Health. Dr. Caron is an accomplished teacher and mentor, having served as an advisor to over 35 graduate, post-graduate, and clinical trainees. She is highly regarded for her impassioned drive for excellence and approach to individualized mentoring and career advising, as recognized by her participation in numerous symposia and articles related to professional development.

Keynote Presentation

Gene Dosage Experiments: How Mouse Genetics Can Model Human Disease

Kathleen Caron, Ph.D.
Department of Cell Biology and Physiology, Department of Genetics and Molecular Biology, UNC McAllister Heart Institute, University of North Carolina School of Medicine, Chapel Hill, NC

Studies in the Caron laboratory have been focused on defining the normal and pathological functions of a small blood protein, called adrenomedullin, which has recently been highlighted as one of the most effective and prognostic clinical biomarkers for heart failure and myocardial infarction. We have used sophisticated gene targeting strategies to develop animal models that have either increases or decreases in adrenomedullin dosage or genetic deficiencies in the G-protein coupled receptor (GPCR) and receptor activity modifying proteins (RAMPs) that transduce adrenomedullin signaling. Today's presentation will use this signal transduction pathway as a backdrop to highlight how specific gene targeting approaches—stemming from initial studies in Dr. Oliver Smithies' laboratory—have paved the way for major advancements in the field of cardiovascular biology. In addition to the commonly utilized gene knockout, other modifications such as single-site integration, gene titration and genetic clamping will be discussed. Our work thus far in animal models has helped to define the biological functions of adrenomedullin under normal and pathological conditions and revealed a critical role for adrenomedullin signaling in lymphatic vascular development and function, which is now being directly translated into novel disease conditions and therapies in humans.

List of Poster Presentations by Predoctoral Students

1. ***Effect of Waterpipe Smoke on the Airway Mucosal Barrier and Innate Defense***
   Sabri Abdelwahab, Boris Reidel, Stephanie Livengood, and Mehmet Kesimer

2. ***Reciprocal Inter-tissue Regulation of Factor XIII-A and -B Subunits Determines Factor XIII Levels in Plasma***
   James R. Byrnes and Alisa S. Wolberg

3. ***AKT Controls the NFkB Pathway by Phosphorylating NEMO at S178***
   Johnny Castillo, Ricardo J. Antonia, and Albert S. Baldwin

4. ***siRNA Screening for Modulators of Programmed Necrosis in Human Cardiomyocytes***
   Matthew Combs, Qiang Zhu, Zachary Opheim, Zhaokang Cheng, and Joan Taylor

5. ***Characterizing the Molecular Mechanisms of Blood Pressure Regulation by GRAF3, a Smooth Muscle Specific RhoGAP***
   Rachel Dee, Xue Bai, Chris Mack, and Joan Taylor

6. ***Dissecting the Function of Classical Cadherins in Stratified Epithelial Morphogenesis.***
   Carlos Patiño Descovich, Kendall Lough, Danielle Spitzer, Michelle Mac, and Scott Williams

7. ***Understanding the Role of Ring1B in Second Heart Field Development***
   Nicole D. Fleming, Li Qian, and Jiandong Liu

8. ***Fibroblasts Induce Subtype-specific Changes in Breast Cancer Cell Behavior and Stromal-epithelial Crosstalk in Association With Epithelial p53 Status***
   Ashley M. Fuller, Rupninder Sandhu, Breanna M.W. Jeffcoat, and Melissa A. Troester

9. ***Role of the Contact System of Coagulation in Acute Liver Failure***
   Michael W. Henderson, R. Pawlinski, and Nigel S. Key

10. ***MuRF1 Deficiency Protects Mice Against Lipopolysaccharide (LPS)-mediated Cardiomyopathy Via the Modulation of Nuclear Factor Kappa B (NF-kB) Pathway***
    Jean Marie Mwiza, Traci L. Parry, and Monte S. Willis

11. ***Focal Adhesion Kinase Promotes Secretion of VEGF and BNP***
    Zachary Opheim, Zhaokang Cheng, Rachel Dee, Michele Casad, Stephanie Gupton, and Joan Taylor

12. ***The Role of Trans-lesion Synthesis in Glioblastoma Resistance to Temozolomide-induced DNA Damage***
    Abigail K. Shelton, Yang Yang, Ryan E. Bash, Cyrus Vaziri, and C. Ryan Miller

13. ***Impact of EGFRvIII and PTEN Deletion Mutations on Response of Ink4a/Arf-null Murine Astrocytes to EGFR Tyrosine Kinase Inhibitors***
Erin Smithberger, Alex R. Flores, Harshil D. Dhruv, Gary L. Johnson, Michael E. Berens, Frank B. Furnari, and C. Ryan Miller

14. ***Metabolic Repatterning in Direct Cardiac Reprogramming***
Haley Vaseghi, Ziqing Liu, Li Wang, Jiandong Liu, and Li Qian

15. ***Elucidating the Effects of HPV Oncogenes in Mouse Epithelium***
Bethany L. Wagner, Kevin M. Byrd, Miranda B. Carper, Antonio L. Amelio, and Scott E. Williams

16. ***GRAF1 is a Novel Regulator of Cardiac Mitophagy***
Qiang Zhu, Kaitlin C. Lenhart, Rachel Dee, Matthew Combs, Chris Mack, and Joan M. Taylor

Effect of Waterpipe Smoke on the Airway Mucosal Barrier and Innate Defense

Sabri Abdelwahab[1,2], Boris Reidel[1,2], Stephanie Livengood[1], and Mehmet Kesimer[1,2]
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Introduction: Waterpipe (hookah, Shisha) is becoming a popular tobacco smoking among adolescents and young adults in United States. In 2016, 5.8% of high school students reported current use of hookahs. Publicly, there is a common misconception that waterpipe smoke is considered a "safer" alternative to cigarette smoking. Its potential health consequences in the airways, however, are poorly understood. Airway mucus acts as the first line of defense against inhaled smoke. Thus, the overarching aim of this study is to investigate the effects of waterpipe smoke on the airway mucosal barrier and epithelial innate host defense. **Methods:** Cultured primary human bronchial epithelial cells (HBECs) were exposed one session of Two-Apple flavor shisha tobacco and air as a control. After acute exposure, the trans-epithelial electrical resistance (TEER) was evaluated to assess cellular integrity. Apical secretions and the basal media were collected and processed to identify and quantitate the proteins secreted using quantitative mass-spectrometry. **Results:** TEER measurement decreased significantly after waterpipe smoke exposure. Label-free proteomic analysis identified more than 1000 proteins in the HBECs apical secretions, including 60 proteins were significantly changed quantitatively in expression when compared to the air group. Furthermore, pathway analysis indicates that proteins related to innate defense/response and oxidative stress are affected by waterpipe exposure. **Conclusions:** Our study indicates that acute waterpipe smoke changes the integrity of the airway epithelial barrier and alters the expression of innate defense proteins, somewhat similar to cigarette smoke exposure. This indicates that waterpipe smoke can pose a potential health risk in the airways.

Reciprocal Inter-tissue Regulation of Factor XIII-A and -B Subunits Determines Factor XIII Levels in Plasma

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Background: Plasma coagulation factor XIII (FXIII) is composed of two zymogen FXIII-A subunits and two carrier FXIII-B subunits (FXIII-A_2B_2). Bone marrow-derived cells produce FXIII-A, whereas hepatocytes synthesize FXIII-B. FXIII-B stabilizes FXIII-A; consequently, deficiency in either subunit is associated with bleeding. Interestingly, FXIII-A-deficient mice and humans have decreased levels of FXIII-B. In humans, therapeutic infusion of recombinant FXIII-A_2 (rFXIII-A_2) increases FXIII-B levels. The mechanism mediating this inter-tissue reciprocal regulation has not been defined. **Objective:** Determine how FXIII-A regulates FXIII-B levels. Methods: FXIII-B protein and mRNA were measured using Western blotting and RT-qPCR, respectively. Liver proteomes were compared using label-free quantitative mass spectrometry and KEGG pathway analysis. **Results:** rFXIII-A_2 treatment increased FXIII-B production in a human hepatocellular carcinoma line (Huh7), but did not promote cell growth or increase FXIII-B stability. *F13a*$^{-/-}$ mice infused with rFXIII-A_2 showed increased FXIII-B levels within 3-6 hours, and by 24 hours, FXIII-B levels were similar to levels in *F13a*$^{+/+}$ mice. *F13b* mRNA isolated from livers of infused mice was not elevated relative to untreated controls, suggesting FXIII-A did not enhance *F13b* gene expression. Proteomic profiling of mouse liver lysates identified unique proteins that were significantly upregulated in both *F13a*$^{+/+}$ and rFXIII-A_2-infused *F13a*$^{-/-}$ mice relative to *F13a*$^{-/-}$ mice. Pathway analysis reveals several of these proteins regulate RNA processing. **Conclusions:** FXIII-A and FXIII-B subunits show reciprocal

regulation in mice and humans. FXIII-B promotes FXIII-A stability in circulation, while FXIII-A enhances FXIII-B production, likely via post-transcriptional processing. Identification of this unique regulatory mechanism exposes newly-recognized inter-tissue crosstalk critical for hemostasis.

AKT Controls the NFkB Pathway by Phosphorylating NEMO at S178

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The Nuclear Factor-kB (NFkB) pathway contributes to development of innate and adaptive immune cells, pathogen recognition, inflammation and other processes. Dysregulation of NFκB has been found to promote cell survival, proliferation, metastasis and angiogenesis in both solid and hematopoietic malignancies. NFkB is known to be required for tumorigenesis downstream of the PI3K/AKT signaling pathway. However, many questions remain unanswered concerning the molecular mechanism of AKT mediated NFkB regulation. AKT shows a strong preference for phosphorylating serines and threonines that are surrounded by the amino acid sequence RxRxxpS/T. Inspection of amino acid sequences of key proteins in the NFkB pathway revealed a putative AKT phosphorylation site (S178) on the NF-kappa-B Essential modulator(NEMO), a key component of IKK, the activating kinase in the NFkB pathway. Indeed, NEMO is recognized by a motif antibody that specifically binds to proteins phosphorylated within putative AKT sites. The antibody fails to recognize NEMO when it is treated with phosphatase or when S178 is mutated to alanine. In addition, treatment with the dual PI3K/mTOR inhibitor BEZ235 decreases binding of this antibody to NEMO. This all suggests that S178 is phosphorylated in cells and that this phosphorylation is PI3K/AKT dependent. Moreover, this phosphorylation site is capable of promoting NFkB as phosphomimetic mutations of S178 increase signal from a NFkB luciferase reporter and mRNA levels of several NFκB target genes. Current work is underway to further characterize the functional consequences of AKT mediated phosphorylation of S178, both for activation of NFkB and for the progression of PI3K/AKT dependent cancer.

siRNA Screening for Modulators of Programmed Necrosis in Human Cardiomyocytes

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Necrosis is a cell death process marked by disintegration of the cellular membrane. Previously thought to be purely incidental, necrosis is now known to encompass molecularly regulated subtypes. Mitochondrial permeability transition-driven regulated necrosis results from calcium overload, however its exact mechanisms remain to be elucidated. As calcium overload is a cause of both ischemic and reperfusion injuries in the heart, discovering ways to prevent calcium overload-induced necrosis would provide a great benefit to public health. Therefore, the objective of this study is to demonstrate that the death of cardiomyocytes due to calcium overload can be reduced by the modulation of programmed necrosis factors. To accomplish this, we performed primary siRNA screens in human bronchial smooth muscle cells to identify potential target genes. We then performed siRNA screening in human cardiomyocytes to validate the previously identified targets. Briefly, we knocked down potential genes via siRNA transfection, induced calcium overload by ionomycin treatment, and then measured necrosis via LDH assay. We identified several genes whose knockdown resulted in higher levels of necrosis and dubbed these genes protectors. We also

identified genes whose knockdown resulted in lower levels of necrosis and dubbed these genes executioners. We conclude that there are several genes which endogenously either inhibit or contribute to calcium overload-induced cardiomyocyte necrosis and that the knockdown of these genes can alter the necrotic response of cardiomyocytes to calcium overload. We are now investigating the function of our most promising targets via the use of agonists and inhibitors in our human cardiomyocyte model.

Characterizing the Molecular Mechanisms of Blood Pressure Regulation by GRAF3, a Smooth Muscle Specific RhoGAP

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We identified a SMC-specific RhoA-GAP (termed GRAF3) that is critical for limiting RhoA dependent SMC contractility and for controlling BP homeostasis. To assess the therapeutic potential for increased GRAF3 to lower blood pressure (BP), we created a tamoxifen-dependent inducible and SMC-specific transgenic mouse model. While basal blood pressure is not significantly altered in GRAF3 overexpressing mice, SMC overexpression of GRAF3 does result in significantly smaller increases in BP following hypertensive agonist treatment (L-NAME) as compared to littermate controls. We also seek to identify critical residues and signaling enzymes that control GRAF3 activity. Interestingly, we found that the GAP activity of GRAF3 is inhibited by an auto-regulatory interaction. Using Pymol and Clus Pro, we developed molecular models of an open/"active" state and a closed/"inactive" state of GRAF3. We identified S152 as a putative post-translational phosphorylation site at the interface between the catalytic and inhibitory domains. Expression of a phospho-deficient (S152A) variant cleared SMC stress fibers more robustly than Wt, while the phospho-mimetic variant (S152D) was unable to clear stress fibers. This indicates that phosphorylation of S152 favors a closed, inactive GRAF3. In vitro kinase assays revealed that S152 is a substrate for p38δ, which is activated by GPCR signaling, suggesting that vasoconstrictors act in part by transiently inactivating GRAF3. Furthermore, we identified Y367 as another potentially important post-translational phosphorylation site that could influence the activation of GRAF3. We have shown that Y376 is a target of Src and FAK kinases, which are known to be activated by elevated intraluminal pressure. Collectively, this work may aid in the development of novel anti-hypertensives.

Dissecting the Function of Classical Cadherins in Stratified Epithelial Morphogenesis

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It is well established that during oriented cell divisions, the positioning of the mitotic spindle is directed by the intracellular scaffolding protein LGN (*Gpsm2*). In the developing epidermis, basal progenitor cells can divide with either a planar (generally thought to be symmetric) or perpendicular (asymmetric) orientation, the latter promotes epidermal stratification. It remains poorly understood how LGN is differentially localized during these divisions. Cadherins are an essential class of cell-cell adhesions that maintain skin integrity at apical junctions through the formation of adherens junctions (AJ). The prototypical E-cadherin (*Cdh1*) has been proposed to influence the cortical localization of LGN by a direct interaction between its juxtamembrane domain (JMD) and the tetra-tricopeptide (TRP) motifs of LGN. A highly-conserved aspartic acid residue (D758) in the JMD is required for this interaction. While this residue is conserved in most other

cadherins, P-cadherin (*Cdh3*) has a glycine substitution which should render it incapable of binding LGN. Indeed, we have found that Pcad and LGN are localized to mutually exclusive cortical domains in basal cells, while Ecad shows significant colocalization with LGN at the apical cell cortex. This suggests that both Ecad and Pcad may have different functions in regulating the localization of LGN. We find that epidermal *Ecad* loss increases the frequency of oblique divisions at the expense of perpendicular divisions, but interestingly, LGN expression appears to be unaffected. This suggests the effect of Ecad on spindle orientation may not be mediated by LGN.

Understanding the Role of Ring1B in Second Heart Field Development

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Heart development requires precise regulation of cardiac progenitor cell (CPC) migration, proliferation, and differentiation in a highly organized spatial and temporal manner. CPCs are grouped into two spatially distinct progenitor pools, namely first heart field (FHF) progenitors and second heart field (SHF) progenitors. During vertebrate heart development, as uncommitted mesodermal cells are specified towards the cardiac lineage, their genomes are epigenetically remodeled to "lock in" the cells to a cardiogenic fate. However, the exact epigenetic mechanism underlying this fate commitment remains elusive. Pioneering work has established that the well-conserved Polycomb group (PcG) proteins, which mediate transcriptional repression via epigenetic silencing of target genes, are essential for regulating various developmental programs, including heart development. A core component of the Polycomb Repressive Complex 1 is Ring1B, which is required for ubiquitination of histone H2A at lysine 119 (H2AK119). A recent study showed that, in contrast to *Ring1b* knockout mice, *ring1b* mutant zebrafish survives early embryonic development but exhibit defective craniofacial and pectoral fin development. In this study, we are investigating the role of Ring1B in early heart development. We have confirmed that *ring1b* mutant hearts fail to loop and appear to be smaller in size compared to the control hearts. Our ongoing studies are using photoconversion experiments to investigate the role of RING1B in SHF progenitor specification. Supported by: American Heart Association Pre-doctoral Fellowship: 17PRE33660515.

Fibroblasts Induce Subtype-specific Changes in Breast Cancer Cell Behavior and Stromal-epithelial Crosstalk in Association With Epithelial p53 Status

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Basal-like breast cancer (BBC), a breast cancer subtype for which targeted therapies are unavailable, accounts for approximately 15% of breast cancer cases and causes disproportionate patient mortality. Clinically relevant BBC behaviors, such as inflammatory cytokine production and cancer cell migration, are strongly promoted by stromal fibroblasts, but little is known about the specific molecular signals that alter stromal-epithelial interactions in the BBC microenvironment. Compared to luminal breast cancers, BBCs are also significantly more likely to exhibit severe deficiencies in p53 pathway function, but little research has addressed how stromal-epithelial crosstalk is altered in association with cancer cell p53 status. To this end, co-cultures of reduction mammoplasty fibroblasts, together with isogenic pairs (p53 wildtype [WT] and knockdown [KD]) of basal-like and luminal breast cancer cells, were used to characterize effects of cancer

cell p53 deficiency on stromal-epithelial interactions. Using a transwell invasion assay, we determined that fibroblasts significantly induced migration of p53 KD, but not p53 WT, BBC cells. This effect was not observed with luminal co-cultures, indicating that p53-dependent cancer cell migration may be specific to BBC. Additionally, whole-genome microarray analyses revealed that IL-17A signaling mediators are upregulated in p53 KD, but not p53 WT, BBC co-cultures. Accordingly, ongoing mechanistic studies are seeking to determine the role of IL-17A signaling in fibroblast-induced, p53-dependent BBC cell migration. Given the high frequency of BBCs with p53 pathway defects, it is critical to understand how cancer cell p53 deficiencies modulate stromal-epithelial interactions, as well as how this crosstalk potentiates effects of p53 loss.

Role of the Contact System of Coagulation in Acute Liver Failure

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Acute liver failure (ALF) is an uncommon, but highly morbid syndrome. Acetaminophen (APAP) toxicity is the single most common cause of ALF in the United States. The acute phase is marked by hepatotoxicity, which may proceed to systemic inflammation, hypotensive shock, and encephalopathy through mechanisms that remain poorly understood. Paradoxically, although patients with APAP toxicity demonstrate these complications much earlier compared to other ALF etiologies, they have the greatest chance of spontaneous survival. Thus, difficulty in choice of an ideal transplant recipient is increased, indicating the necessity for a selective biomarker. Attenuation of the TF/factor VIIa (extrinsic) pathway of coagulation has been shown to offer protection within the first 24 hours in animal models. We are testing the hypothesis that the CS plays a role in ALF progression, may provide novel biomarkers for severity and selection of the need for liver transplant, and may be pharmacologically inhibited to limit disease progression. Animal studies using knockout animals deficient in Factor XII and high molecular weight kininogen demonstrated reduced alanine aminotransferase, and reduced hepatocellular injury. Analysis of human ALF samples for presence of FXIIa, FXIa or kallikrein complexed with their common endogenous inhibitor, C1- inhibitor, using novel ELISAs have demonstrated that the contact system is activated *in vivo*, and at levels 8-fold greater than normal controls. These studies will increase our understanding of the pathogenesis of ALF, and offer novel bench-to-bedside approaches to prognostication and treatment.

MuRF1 Deficiency Protects Mice Against Lipopolysaccharide (LPS)-mediated Cardiomyopathy Via the Modulation of Nuclear Factor Kappa B (NF-kB) Pathway

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Sepsis induced-cardiomyopathy (SICM), the major cause of cardiovascular problems in ICU, leads to higher mortality in septic patients. The pathogenesis of SICM is not completely understood. The peroxisome proliferator-activated receptor alpha (PPARα) isoform, which is highly expressed in the heart, has been shown to competitively inhibit NF-kappaB mediated inflammation. NF-κB is a transcriptional activator of genes involved in sepsis induced inflammatory response. We recently demonstrated that E3 ubiquitin ligase muscle ring finger-1 (MuRF1) inhibit PPARα expression by tagging it for nuclear export and ultimate degradation by the ubiquitin proteasome system (UPS). Here, we investigated the role of MuRF1 in LPS induced cardiac dysfunction. MuRF1-/- and MuRF1+/+ mice were injected with 2 mg/kg regimen of LPS

for up to 72 hours. Transthoracic echocardiography was used to measure heart function at baseline, 4, 24, 48 and 72 hrs post-LPS IP injection. We used western blot to determine levels of pro-inflammatory mediators in these mice which were sacrificed at each of the 5 time points post-LPS injection. We demonstrated that MuRF1-/- LPS treated mice were protected from cardiac dysfunction. The wild type mice showed a sustained worse cardiac dysfunction, demonstrated by worse systolic function, posterior & septal wall thinning and cardiac atrophy. Furthermore, MuRF1-/- hearts had significant decreases in IL1β, IκB-alpha, P-IKK, TAK1 and p-IκB at 4hrs post-LPS treatment, consistent with inhibition of the level of IKK. These results together demonstrate that MuFR1 modulate the early inflammatory response in part via the NF-κB pathway, making MuRF1 a muscle specific anti-inflammatory therapeutic target.

Focal Adhesion Kinase Promotes Secretion of VEGF and BNP

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The heart releases various paracrine and endocrine factors such as VEGF and ANP, which regulate cardiac homeostasis and participate in disease pathogenesis. Although cardiomyocytes secrete small peptides, the process by which they are trafficked to the plasma membrane and secreted remains unknown. Recent studies by the Taylor lab implicate focal adhesion kinase (FAK) as a candidate in VEGF secretion as FAK regulates angiogenesis in the developing heart and provides protection during ischemia reperfusion injury, an event associated with increased blood vessel density after injury. These findings lead us to hypothesize that FAK promotes secretion of VEGF and other cardiopeptides to confer cardioprotection. Therefore, the objectives of this study were to determine if FAK regulates secretion of VEGF and ANP, and to uncover the trafficking machinery responsible for VEGF and ANP release. We found that overexpression of FAK increases ischemia induced VEGF secretion in neonatal rat ventricular myocytes (NVRMs). Furthermore, pharmacological inhibition of FAK reduces phenylephrine dependent ANP release in NVRMs and these changes were not due to an increase in cardiopeptide production. Knockdown of V-SNARES VAMP 2 and 3 significantly reduced secretion of NVRMs exposed to hypoxia. Moreover, knockdown of VAMP 2 significantly decreased phenylephrine stimulated ANP release in NVRMs. Interestingly, using TIRF microscopy we discovered mobility of secretory VAMP2 and VAMP3 -labeled vesicles requires FAK activity. Finally, VAMP2/3 vesicle dynamics depend on FAK stabilization of microtubules for proper trafficking. Collectively, our data suggests ANP and VEGF traffic in VAMP 2 and 3 labelled vesicles respectively and their secretion is dependent upon FAK activity.

The Role of Trans-lesion Synthesis in Glioblastoma Resistance to Temozolomide-induced DNA Damage

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Glioblastoma (GBM) is the most common and aggressive adult brain tumor. Survival is only 12-15 months after therapy, including resection, radiation, and temozolomide (TMZ) chemotherapy. Intrinsic and acquired TMZ resistance are nearly universal. Intrinsic TMZ resistance is mediated by methylguanine methyltransferase (MGMT)-mediated repair of methyl-DNA adducts. Mismatch repair leads to acquired

resistance. The role of trans-lesion synthesis (TLS) remains unknown. We examined the role of TLS in response to TMZ using cultured normal human astrocytes immortalized via HPV E6/E7 and hTERT (NHA) and Ras-transformed NHA (NHA-Ras). Immunoblots in both lines showed that TMZ induced markers of TLS: RAD18, polymerase eta (Polη), and mono-ubiquitinated PCNA (ubPCNA). TMZ induced temporal increases in ubPCNA and DNA damage markers γH2AX and pChk1 over 24 hours in NHA-Ras. RAD18 depletion via RNAi sensitized NHA-Ras, but not NHA, cells to TMZ by clonogenic assay. We next examined proliferation and TMZ toxicity by MTS assay in a panel of RAD18-expressing human GBM cell lines: U87MG, U251MG, U373MG, D54MG, LN18, and LN229. Doubling times ranged from 0.9-1.5 days. A 16.4-fold difference in TMZ IC_{50} was evident between the most sensitive (D54MG, 5.4 μM) and most resistant (U373MG, 88.7 μM) lines. LN18 was exquisitely resistant (IC_{50} ~ 654 μM), likely due to high MGMT expression. Co-IP showed that TMZ induced Polη interaction with ubPCNA in NHA-Ras cells. These data suggest that the TLS pathway is activated as a resistance mechanism to TMZ. Overall, the TLS pathway represents a potential novel druggable target for treatment of TMZ-resistant GBM.

Impact of EGFRvIII and PTEN Deletion Mutations on Response of Ink4a/Arf-null Murine Astrocytes to EGFR Tyrosine Kinase Inhibitors

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Epidermal growth factor receptor (EGFR) represents the most attractive target for personalized glioblastoma (GBM) treatment due to its frequency and specificity for the disease, and the availability of multiple targeted tyrosine kinase inhibitors. Despite its therapeutic attractiveness, several EGFR-targeted tyrosine kinase inhibitors (TKI) have failed clinically, partly due to multiple molecular resistance mechanisms. To dissect the genetic contributions to EGFR TKI resistance, we examined the sensitivity of Ink4a/Arf (CDKN2A)-null murine astrocytes engineered with EGFRvIII, the most common activating mutation in GBM (deletion of exons 2-7 in the extracellular domain), to a panel of EGFR TKI *in vitro*. We examined the role of PTEN deletion on drug sensitivity and found that EGFRvIII conferred sensitivity to 4/5 drugs (ΔIC_{50} 1.5-6.5-fold) in the presence of wild-type Pten and 5/5 (ΔIC_{50} 1.5-78.0-fold) with Pten loss. Conversely, Pten deletion conferred resistance to 4/5 drugs (ΔIC_{50} 5.6-17.7-fold) in Ink4a/Arf-null astrocytes with wild-type Egfr and 2/5 (ΔIC_{50} 1.4-5.4-fold) in cells with EGFRvIII. Ink4a/Arf-null, EGFRvIII-mutated astrocytes developed cross resistance to the other 4 TKI (ΔIC_{50} 2.6-11.0-fold) when grown continuously with increasing gefitinib (≤2μM). We have previously shown that dynamic kinome responses may be responsible for TKI resistance. We are currently using chemical proteomics to assess the functional state of the kinomes of these non-germline genetically engineered mouse (nGEM) models in the presence (dynamic) and absence (baseline) of EGFR TKI. Thus, functional kinome analysis using targeted EGFR TKI will help define the kinase networks required for EGFRvIII-driven GBM pathogenesis and may aid in the identification of novel treatment combinations.

Metabolic Repatterning in Direct Cardiac Reprogramming

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Direct cardiac reprogramming is a promising approach to cardiac regeneration. Fibroblasts are converted directly into induced cardiomyocytes (iCMs) *in vitro* and *in vivo* through overexpression of three cardiac

lineage transcription factors – Gata4, Mef2c, and Tbx5. However, little is known about the sequence of events or mechanisms regulating cellular changes during this transdifferentiation process. Here we characterize mitochondrial and metabolic repatterning during direct cardiac reprogramming. We examined changes in gene transcription during iCM reprogramming using single cell transcriptomics profiling and found that genes associated with mitochondria and oxidative phosphorylation are significantly upregulated. We subsequently examined mitochondrial changes during reprogramming and found that iCM reprogramming increases cellular mitochondria quantity and respiratory capacity. We then inhibited mitochondrial dynamics using RNA interference knockdown of key regulators and found that inhibiting mitochondrial fission increases reprogramming efficiency. Moreover, inhibiting mitochondrial fission further increases mitochondrial content and respiratory capacity during reprogramming. Future studies will explore the role of mitochondrial dynamics in direct cardiac reprogramming. Understanding mitochondrial and metabolic changes during reprogramming has potential to improve the reprogramming process for therapeutic applications.

Elucidating the Effects of HPV Oncogenes in Mouse Epithelium

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Head and neck squamous cell carcinomas (HNSCC) arise mainly through infection with human papilloma virus (HPV) or the accumulation of mutations in the oral epithelium caused by tobacco and alcohol exposure. Using a novel murine model of HPV which expresses HPV oncogenes E7 and E6 under the control of Cre recombinase, we investigated HPV-driven transcriptional changes in neonatal and adult epithelium. In addition to validating the differential regulation of known E7 and E6 targets, we identified additional cell biological changes in polarity, differentiation, and cell adhesion molecules. The altered transcriptional profiles observed through quantitative PCR and RNA sequencing were corroborated with an interrogation of expression of the targets at the protein level using immunohistochemistry. Together, these data provide a better understanding of the cellular changes induced by HPV oncoproteins, which can be used to investigate genetic mechanisms of HNSCC and identify putative therapeutic targets. Supported by: Robert H. Wagner Scholar Award (B.W.), Sidney Kimmel Foundation Kimmel Scholar Award SKF-15-065 (S.W.), R21-DE025725 (S.W. and A.A.), T90-DE021986 (M.C.), R00-CA157954 (A.A.).

GRAF1 is a Novel Regulator of Cardiac Mitophagy

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Mitochondria act as a key nodal point in mediating cardiac function. Cell death due to mitochondria dysfunction underlies numerous heart diseases. However, the mechanism underlying finely tuned regulation of mitochondria biogenesis, maintenance and turnover remains unclear. The goal of this study is to elucidate the regulatory mechanism of mitochondria homeostasis in heart. We found that GRAF1 (GTPase regulator associated with focal adhesion kinase-1) localizes to damaged mitochondria. Attenuation of GRAF1 leads to mitochondria depolarization as well as accumulation of mitochondrial ROS in cardiomyocytes, suggesting that GRAF1 is required for healthy mitochondria. Next, we assessed the role of GRAF1 in mitochondria turnover (mitophagy) and found that attenuation of GRAF1 leads to dramatic accumulation of mitochondria in neonatal rat cardiomyocytes (NRCMs) and reduces LC3 II flux, indicating that GRAF1 is required for

macroautophagy and mitophagy. Accumulation of damaged mitochondria results in cellular death. GRAF1 knockdown in cardiomyocytes induces apoptosis. Our study further shows that GRAF1 can specifically interact with LC3. Consistently, while viable, GRAF1 hypomorphic mice exhibit cardiac mitochondria defects as assessed by TEM and treatment with isoproterenol induces cardiomyopathy in these mice. Collectively, our study indicates that GRAF1 is a novel regulator of cardiac mitophagy and is essential for maintenance of mitochondrial homeostasis in cardiomyocytes.

List of Poster Presentations by Postdoctoral Fellows and Clinical Fellows

17. ***RhoGap GRAF3 Serves as a Blood Volume-sensitive Rheostat to Control Smooth Muscle Contractility and Blood Pressure***
Xue Bai, Kevin Mangum, Masao Kakoki, Oliver Smithies, Christopher P. Mack, and Joan M. Taylor

18. ***Defining Mucin-mucin and Mucin-protein Interactions in the Airways: The Mucin Interactome***
Jerome Carpenter, Giorgia Radicioni, and Mehmet Kesimer

19. ***Defining the Role of Exosomes in Airway Biology and Epithelial Remodeling***
Richa Gupta, Giorgia Radicioni, Hong Dang, Piotr Mieczkowski, John Sheridan, Scott H. Randel, and Mehmet Kesimer

20. ***Human Pancreatic Tumors Grown in Mice Release Tissue Factor-positive Microvesicles That Increase Venous Clot Size***
Yohei Hisada, Cihan Ay, Alyson C. Auriemma, Brian C. Cooley, and Nigel Mackman

21. ***Airway Mucin Concentration as a Biologic Hallmark of Chronic Bronchitis***
Giorgia Radicioni, Amina A. Ford, Agathe Ceppe, and Mehmet Kesimer

22. ***E-Cigarette Use Causes a Unique Response in the Lung Involving Increased Neutrophilic Activation and Altered Mucin Secretion***
Boris Reidel, Giorgia Radicioni, Phillip Clapp, Amina A. Ford, Sabri Abdelwahab, Meghan E. Rebuli, Prashamsha Haridass, Neil E. Alexis, Ilona Jaspers, and Mehmet Kesimer

23. ***Exome Sequencing in Conjunction With Cytogenetic Analysis by FISH for Diagnosis of Fetal Malformation***
Natasha T. Strande, Kelly Gilmore, Ann Katherine Foreman, Alexandra Arreola, Daniel S. Marchuk, Bradford Powell, Chris Bizon, Phillips Owen, Kirk Wilhelmsen, Jonathan S. Berg, Kathleen A. Kaiser-Rogers, Karen E. Weck, and Neeta L. Vora

24. ***MuRF1-Related Metabolic Alterations in HL-1 Cardiomyocyte Induced By Stretch***
Wei Tang, Jessica E. Rodríguez, Amro Ilaiwy, and Monte S. Willis

RhoGap GRAF3 Serves as a Blood Volume-sensitive Rheostat to Control Smooth Muscle Contractility and Blood Pressure

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Vascular resistance is a major determinant of BP and is controlled, in large part, by RhoA-dependent smooth muscle cell (SMC) contraction. Our previous studies indicate that GRAF3 is a critical regulator of RhoA in vascular SMC. The elevated contractile responses we observed in GRAF3 deficient vessels coupled with the hypertensive phenotype provided a mechanistic link for the hypertensive locus recently identified within the GRAF3 gene. On the basis of our previous findings that the RhoA signaling axis also controls SMC contractile gene expression and that GRAF3 expression was itself controlled by this pathway, we postulated that GRAF3 serves as an important counter-regulator of SMC phenotype. Indeed, our new findings presented herein indicate that GRAF3 expression acts as a pressure-sensitive rheostat to control vessel tone by both reducing calcium sensitivity and restraining expression of the SMC-specific contractile proteins that support this function. Collectively, these studies highlight the potential therapeutic value of GRAF3 in the control of human hypertension.

Defining Mucin-mucin and Mucin-protein Interactions in the Airways: The Mucin Interactome

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Objectives: The biophysical/biochemical properties of mucus allow it to function as a transportable barrier. In mucoobstructive diseases, however, mucus becomes dysfunctional; it's increased adherence to the epithelial surface, as well as visco-elasticity leads to the formation of plugs and plaques, which impair lung function. The interactions between mucins and the proteins of the mucin interactome play a major role in determining the biophysical properties of mucus and are important for having healthy, functional mucus. **Methods:** Mucins and their interacting proteins were gently isolated and their interaction were further monitored and characterized by AFM/EM and Quartz Crystal Microbalance with Dissipation (QCMD). **Results:** AFM images indicated, DMBT1 and membrane bound mucins were identified in the mucus network, together with gel-forming mucins. Furthermore the respective interactions between DMBT1, Galectin-3 and LPLUNC1 and MUC5B were quantified using QCMD. Galectin-3 increased both the viscosity and elasticity of the mucin layer while reducing the layer thickness. DMBT1 was shown to increase the viscosity of the layer, but also increased the total dissipation. **Conclusion:** The data indicates mucins alone aren't sufficient in accounting for the biophysical properties of mucus. In characterizing the mucin-mucin and mucin-protein interactions, we further our understanding of what defines a functional mucus, as well as our understanding of the properties of diseased mucus. The observation of membrane bound mucins interacting with the gel forming mucins suggests a possible new mechanism for mucus/epithelial adhesion, which plays a role in many of the mucoobstructive diseases.

Defining the Role of Exosomes in Airway Biology and Epithelial Remodeling

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Introduction: To understand the role of exosomes in airway remodeling through cell-cell communication in lungs we utilized two distinct airway cultures, HTBE and Calu-3. After controlled, in-vitro, inter-cellular exosomal transfer, a comprehensive proteomic and genomic characterization of cell secretions and exosomes is explored. **Methods:** HTBE cells were treated with Calu-3 derived exosomes, and Calu3 cells were treated with HTBE exosomes for three days. Apical secretions before and after treatments were collected and subjected to proteomic analysis. Also, exosomal cargo (protein and miRNA) from both cultures were analyzed. **Results:** Proteomic analysis of the secretions indicated that the two cell cultures have distinct secretomes. The expression of gel-forming mucins, MUC5AC and MUC5B, and their ratio were significantly higher in Calu-3 secretions (15-fold). Innate immune proteins, FCGBP, CEACAM 1, PIGR, DMBT1, were significantly higher in Calu-3. After the exosome exchange, these profile dramatically altered. Proteins including ceruloplasmin, DMBT1, Complement C3, MUC5B and MUC5AC, Galectin-3-binding protein were significantly upregulated in HBE secretion. About 240 miRs were differentially expressed in HBE and Calu3 exosomes. Pathway analysis indicated that miRNAs related to cancer metastasis, inflammation and mucin production, miR-19a, miR-19b-3p, miR-14, miR-200c, miR-149-3p, miR-4497, miR-6752-5p were more abundantly represented in the Calu-3 cells. After the exosomal transfer, about 90 miRs related to ciliogenesis (miR-34, miR-449) and C-myc and NF-kB pathway (mir-31-5p, miR-27b-3b, miR-21, miR-34, miR-100-5p, miR-192-5p) were upregulated in HBE secretion. **Conclusion:** Our data suggests that exosomal content can be transferred between airway cells, can modulate cell microenvironment, and play a role in airway remodeling during inflammation.

Human Pancreatic Tumors Grown in Mice Release Tissue Factor-positive Microvesicles That Increase Venous Clot Size

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Background: Pancreatic cancer patients have a high rate of venous thromboembolism. Human pancreatic tumors and cell lines express high levels of tissue factor (TF) and release TF-positive microvesicles (TF+MVs). In pancreatic cancer patients, tumor-derived TF+MVs are present in blood and increased levels are associated with venous thromboembolism and decreased survival. Previous studies have shown that mice with orthotopic human or murine pancreatic tumors have circulating tumor-derived TF+MVs, an activated clotting system, and larger clots than controls in an inferior vena cava stenosis model. These results suggest that TF+MVs contribute to venous thrombosis. However, the specific role of tumor-derived, TF+MVs in venous thrombosis in mice has not been determined. **Objectives**: We tested the hypothesis that tumor-derived, TF+MVs enhance venous thrombosis in mice. **Methods**: We determined the contribution of TF+MVs derived from human pancreatic tumors grown orthotopically in nude mice to venous clot formation by using an anti-human TF monoclonal antibody. We used an inferior vena cava stasis model of

venous thrombosis. **Results:** Tumor-bearing mice had significantly larger venous clots than control mice. Clots from tumor-bearing mice contained human TF, suggesting the incorporation of tumor-derived MVs. Importantly, administration of an anti-human TF monoclonal antibody reduced clot size in tumor-bearing mice but did not affect clot size in control mice. **Conclusions**: Our results indicate that TF+MVs released from orthotopic pancreatic tumors increase venous thrombosis in mice. This new model may be useful in evaluating the role of different factors in pancreatic cancer-associated venous thrombosis.

Airway Mucin Concentration as a Biologic Hallmark of Chronic Bronchitis

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Background: Chronic obstructive pulmonary Disease (COPD) is characterized by chronic bronchitis (CB) and emphysematous components. Mucins are large, heavily glycosylated proteins and its concentration in the airways is a key variable that mediates bronchial mucus flow: for this reason, we postulated that raised mucin concentration maybe a biochemical feature of CB in COPD subjects. **Methods:** Total mucin concentration was measured by size-exclusion chromatography/differential refractometry in 917 SPIROMICS subjects. The two major airway gel-forming mucins (MUC5AC and MUC5B), were quantified by a mass spectrometry Parallel Reaction Monitoring (PRM) labelled quantitation technique in 148 subjects. Both measurements were related to the GOLD stage classification, which correlates the severity with the FEV_1 value and to the CB symptom scores. **Results:** Total mucin concentration is significantly associated with patient perception of phlegm production and characteristics, GOLD stage defined disease severity, disease etiology (smoking and asthma history) and current questionnaire based diagnosis of CB. Absolute concentrations of individual mucins MUC5AC and MUC5B increases 10 times and 3 times respectively in current or former smokers with severe COPD. Receiver-operating-characteristic curve analysis of the association between total mucin concentration and a diagnosis of chronic bronchitis yielded areas under the curve of 0.72 for the SPIROMICS cohort and 0.82 for the independent cohort. **Conclusions:** Airway mucin concentrations may quantitate a key component of the chronic bronchitis pathophysiologic cascade that produces sputum and mediates disease severity. Studies designed to explore total mucin concentrations in sputum as a diagnostic biomarker and therapeutic target for chronic bronchitis appear to be warranted.

E-Cigarette Use Causes a Unique Response in the Lung Involving Increased Neutrophilic Activation and Altered Mucin Secretion

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Rationale: The detrimental health effects of cigarette smoking have been well established. However, little is known about the potential adverse health effects of e-cigarette use. In this study we assessed the effects of e-cigarette use on the airway secretomes of e-cigarette users versus cigarette and non-smokers. **Methods:** Induced sputum samples from cigarette smokers, e-cigarette users and non-smokers were analyzed by quantitative proteomics and the total mucin and individual MUC5AC/MUC5B concentrations were determined by light-scattering/refractometry and labeled mass spectrometry respectively. Isolated peripheral neutrophils from tobacco product users and non-smokers were analyzed for NET-formation using phorbol

ester (PMA) stimulation. **Results:** Sputum samples from e-cigarette users exhibited significant increases in smoke exposure marker proteins, such as aldehyde detoxifying enzymes and factors related to oxidative stress response. Innate defense proteins associated with COPD, such as elastase and matrix metalloproteinase 9 were significantly elevated in e-cigarette users, as were neutrophil extracellular trap (NET)-related proteins, such as myeloperoxidase, protein disulfide isomerase, azurocidin, and protein-arginine deiminase 4, despite no significant elevation in neutrophil cell numbers. Peripheral neutrophils from e-cigarette users showed increased NETosis. In addition, a compositional change of airway mucus, i.e., an elevated ratio of mucin MUC5AC/MUC5B, was observed in cigarette smokers and e-cigarette users. **Conclusions:** Our results indicate that e-cigarette use distinctly alters the airway secretome, inducing similar and unique changes compared to cigarette smoking. Unique to e-cigarette users were elevated levels of neutrophil- and epithelia-derived innate defense proteins, including proteins related to NET formation, and neutrophil activation. These results challenge the concept that switching from cigarettes to e-cigarettes is necessarily a healthier alternative. Research Funding Source: TCORS P50HL120100.

Exome Sequencing in Conjunction With Cytogenetic Analysis by FISH for Diagnosis of Fetal Malformation

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Congenital malformations occur in 2-4% of all births and are the leading cause of death in the perinatal period. Genomic abnormalities are associated with congenital malformations and can be detected prenatally using karyotype and microarray analysis, but are only present in 10% of fetal anomalies. Diagnostic exome sequencing (ES) of affected fetuses with normal karyotype and microarray analysis has recently been employed with diagnostic rates ranging from 10-60%. We utilized ES in conjunction with cytogenetic analysis by FISH (fluorescent in situ hybridization) to evaluate a fetus with structural abnormalities suggestive of short-rib polydactyly identified by second trimester ultrasound. Amniocentesis followed by karyotype revealed a normal 46,XY fetus. Retrospective ES of the frozen amniocytes was performed in conjunction with parental samples. An in-house variant analysis infrastructure was used to annotate and prioritize variants that were either de novo or inherited *in trans*. Exome data was also evaluated for copy number variation using an algorithm to compare depth of coverage across each of the exomes. Trio exome analysis identified a heterozygous maternally inherited likely pathogenic missense variant in the *DYNCH21* gene located on chromosome 11, which is associated with autosomal recessive short-rib polydactyly. A second sequence variant in *DYNCH21* was not found; however a paternally derived 20 exon duplication of *DYNCH21* was detected with our copy number algorithm. Subsequent FISH analysis of the father's sample confirmed the duplication occurs on chromosome 11. Furthermore, FISH probes flanking the duplicated region of *DYNCH21* co-localized with the enhanced signal for *DYNCH21* duplication, suggesting an intragenic duplication. These results are consistent with compound heterozygous mutation of *DYNCH21* and a diagnosis of short-rib polydactyly. Our results illustrate the utility of ES to identify both single nucleotide variants and copy number variation in association with fetal anomalies. This case further demonstrates the value of both molecular and cytogenetic techniques in identifying the etiology of various congenital malformations facilitating counseling about accurate recurrence risks and genetic testing options.

MuRF1-Related Metabolic Alterations in HL-1 Cardiomyocyte Induced By Stretch

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Objective. The giant myofibrillar protein titin acts as a stretch sensor and the muscle-specific ubiquitin ligase MuRF1 binds to the titin kinase pseudokinase to inhibit the localization and activity of the SRF transcription factor. While we have recently identified how the closely related MuRF1 family member inhibits PPARα at the molecular level, a link between stretch and MuRF1 activity has not previously been established. **Methods.** HL-1 cells plated on rubber bottom plates were transduced with Ad.shRNA MuRF1 (or Ad.shRNA Scramble control) to knock-down MuRF1 protein to <25% of controls and subjected to 15% biaxial stretch at 1 Hz using. In parallel, we collected cell media and performed GC-MS non-targeted metabolomics to identify the role of MuRF1 in the dynamic metabolic changes in cardiomyocytes. **Results.** MuRF1 knock-down resulted in a stretch-induced increase in nuclear localization by immunofluorescence microscopy, which paralleled increases in nuclear PPARα activity (but not PPARα protein levels). Non-targeted metabolomics analysis identified eight significantly altered metabolites by ANOVA, including metabolites involved in the citric acid cycle (citric acid/isocitric acid, glutamic acid), the Ehrlich amino acid degradation pathway (2-ketovaline, 2-ketoleucine), and uracil/arginine metabolism (uracil, putrescine) in addition to 6-aminohexanoic acid/delta-aminolevulinic acid (DALA) and putrescine. MuRF1 knockdown altered all of these metabolites significantly except DALA and putrescine. **Conclusion.** These studies identify MuRF1's role in inhibiting PPARα during stretch, by maintaining its localization (and activity) out of the cell during biaxial cyclic stretch, and give insight into the possible link between the phenotypic stretch seen in heart failure and the metabolic adaptations.

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W.B. Coleman and *J.W. Homeister*