American Thoracic Society

ASSEMBLIES

GROVER CONFERENCE

2019

September 4-8, 2019

Lost Valley Conference Center, Sedalia, CO

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THE PROGRAM

About the Program

Since its inauguration in 1984, the 2019 Grover Conference will be the 19th in this series, representing the longest-standing conference on Pulmonary Circulation. Today it remains the principal conference for pulmonary vascular function, directly related to the interests of the ATS. Relatively small groups of attendees and highly focused topics facilitate maximal contact for scientific discourse. The seclusion of the Conference Center in Sedalia, CO provides the best opportunity for undisturbed exchange of ideas at both formal sessions and informal meetings at the conference center. The meeting is open to all interested scientists and clinician-scientists. As with past Conferences, this Conference will consist of a productive mix of young and senior scientists. Although the total number of participants is limited, we anticipate that the overall conference participants, including speakers and attendees, will be diverse and involve participants drawn from many ATS Assemblies.

Program Objectives

The molecular tools available to interrogate the biology of the pulmonary vasculature is ever more powerful. Yet, changes in the transcriptome of the lung across organismal life span remain incomplete. The present conference will seek to wed the biology of pulmonary vasculature with novel transcriptomic based approaches. Participants will gain new insights into the tools that can now be brought to bear on the cellular constituents of the pulmonary vasculature.

Learning Objectives

At the conclusion of this program, participants should be able to:

1. Learn about the influence of epigenetic, maternal, and neonatal factors on pulmonary vascular development across the lifespan.
2. Learn the power, promise and limitations of applying transcriptomic based approaches to pulmonary vascular research.
3. Recognize the implications of hypoxia relative to the regulation of pulmonary vascular tone and lung development.
4. Consider the implications of translating gene editing technologies from bench to bed.
5. Recognize the implications of cell-cell communications in physiologic and pathophysiologic pulmonary vascular development.

Who Should Attend

Pulmonary vascular biologists, molecular biologists, computational biologists, drug developers, pulmonary immunologists, neonatologists, translational research scientists, clinical researchers, stem cell biologists.

PROGRAM COMMITTEE

Steven H. Abman, MD, Co-Chair, Organizer
David N. Cornfield, MD, Co-Chair, Organizer
Sebastien Bonnet, PhD, MSc
Elena A. Goncharova, PhD

SPEAKERS AND SESSION CHAIRS

Cristina Alvira, MD, Stanford University, Stanford, CA

Vineet Bhandari, MD, Drexel University, Philadelphia, PA

Peter F. Carmeliet MD, PhD, University of Leuven, Leuven, Belgium

Wendy K. Chung, MD, PhD, Columbia University, New York City, NY

Harry (Hal) C. Dietz, MD, PhD, Johns Hopkins University, Baltimore, MD

Anne Eichmann, PhD, Yale University, New Haven, CT

Marlowe Eldridge, MD, University of Wisconsin-Madison, Madison, WI

Csaba Galambos, MD, PhD, University of Colorado

William T. Gerthoffer, PhD, Mobile, AL

Mark T. Gladwin, MD, University of Pittsburgh, Pittsburgh, PA

Paul Hassoun, MD, Johns Hopkins University, Baltimore, MD

Luisa Iruela-Arispe, MSc, PhD, University of California Los Angeles, Los Angeles, CA

Jeffrey Kahn, PhD, MPH, Johns Hopkins University, Baltimore, MD

Stella Kourembanas, MD, Harvard University, Cambridge, MA

Maya Kumar, PhD, Stanford University, Boulder, CO

Phil Levy, MD, Boston Children's Hospital, Boston, MA

Adam Lewandowski, PhD, Oxford University, Oxford, UK

Wolfgang Liedtke, MD, PhD, Duke University, Durham, NC

Brad A. Maron, MD, Harvard University, Cambridge, MA

Karen Mestan, MD, Northwestern University, Evanston,IL

Mark Nicolls, MD, Stanford University, Stanford, California

Laura E. Niklason, MD, PhD, Yale University, New Haven, CT

MA Pasha, PhD, Delhi, India, Mobile, AL

Shahin Raffi, MD, Cornell University, Ithaca, NY

Usha Raj, MD, University of Illinois Chicago, Chicago, IL

Erinn Rankin, PhD, Stanford University, Stanford, CA

Larissa Shimoda, PhD, MS, Baltimore, MD

Kurt Stenmark, MD, University of Colorado, Minneapolis, MN

Troy Stevens, PhD, University of South Alabama

Xin Sun, PhD, University of California San Diego, San Diego, CA

Elizabeth Taglauer, MD, PhD, Harvard University, Cambridge, MA

Cormac T. Taylor, PhD, University College Dublin, Dublin, Ireland

Bernard Thebaud, MD, PhD, Ottawa Hospital Research Institute, Ottawa, Canada

Jeffrey A. Whitsett, MD, Cincinnati Children's Hospital, Cincinnati, OH

Joshua Wythe, PhD, Baylor College of Medicine, Houston, TX

Jason X. J. Yuan, MD, PhD, University of California, San Diego, CA

Jarod Zepp, PhD, University of Pennsylvania, Philadelphia, PA

2019 GROVER CONFERENCE
On the Pulmonary Vasculature in Development, Injury and Repair

COURSE SCHEDULE

Wednesday, September 4, 2019

12:00 pm **Arrivals**

6:00 pm **Welcome Reception and Dinner**

7:15 pm **Welcome and Introduction**
David N. Cornfield MD, Co-Chair, Organizer
Steven H. Abman, MD, Co-Chair, Organizer

7:30 pm **Ethical Implications of Gene Editing in Human Biology**
Jeffrey Kahn, PhD, MPH, Johns Hopkins University

Thursday, September 5, 2019

Session I: Pulmonary Vascular Development, Passenger or Driver?
Moderator: Steven Abman, MD

7:00 am **Breakfast**

7:45 am **The Placenta – Lung Vascular Interface**
Karen Mestan, MD, Northwestern University

8:15 am **The Placental-Pulmonary Connection: Nature's Original Respiratory Circuit**
Elizabeth Taglauer, MD, PhD, Harvard University

8:45 am **Genetic Mechanism of Pediatric Pulmonary Vascular Disease**
Xin Sun, PhD, University of California San Diego

9:15 am **Break (15 min)**

9:30 am **Robyn Barst Lecture: Genetics of Pulmonary Vascular Disease in Children**
Wendy K. Chung, MD, PhD, Columbia University

Session II: Pulmonary Vascular Disease Across the Lifespan- "Canaries in a Coal mine?
Moderator: Brad Maron, MD

10:15 am **Late pulmonary vascular disease in children after preterm birth**
Phil Levy, MD, Boston Children's Hospital

10:45 am **Pulmonary Vascular and Right Ventricular Dysfunction in Adults Born Preterm**
Marlowe Eldridge, MD, University of Wisconsin-Madison

11:15 am **Jack Reeves Lecture: Adult Cardiac and Systemic Vascular Health After Preterm Birth**
Adam Lewandowski, PhD, Oxford University

12:00 pm **Lunch and Afternoon Break**

COURSE SCHEDULE

Session III: Basic Mechanisms of Lung Vascular Development, Injury and Repair: Human and Mouse
Moderator: Usha Raj, MD

3:15 pm **Lung Map: Insights into lung vascular development and repair**
Jeffrey A. Whitsett, MD, Cincinnati Children's Hospital

3:45 pm **Epithelial-mesenchymal interactions during lung vascular development**
Jarod Zepp, PhD, University of Pennsylvania

4:15 pm **Pulmonary endothelial heterogeneity during postnatal lung growth at single cell resolution**
Cristina Alvira, MD, Stanford University

4:45pm **Break (15min)**

5:55 pm **Guidance of vascular barrier formation**
Anne Eichmann, PhD, Yale University

5:25 pm **Role of miRNAs during Lung Vascular Development and Injury**
Vineet Bhandari, MD, Drexel University

6:00 pm **Dinner**

Friday, September 6, 2019

Session IV: Systemic Disease and Lung Vascular Injury
Moderator: David Cornfield MD

7:00 am **Breakfast**

7:45 am **The Terry Wagner Lecture: Angiogenesis Revisited: Role and (Therapeutic) Implications of Endothelial Metabolism**
Peter F. Carmeliet MD, PhD, University of Leuven, VIB, Belgium

8:30 am **Notch3-marked subpopulation in the artery wall is the cell of origin for occlusive vascular lesions in a novel model of pulmonary hypertension**
Maya Kumar, PhD, Stanford University

9:00 am **Break (15 min)**

9:15 am **At the border of normal and PH: Focusing on disease inception**
Brad A. Maron, MD, Harvard University

10:15 am **Mechanisms and new therapies for pulmonary hypertension in the setting of heart failure with preserved ejection fraction (PH-HFpEF)**
Mark T. Gladwin, MD, University of Pittsburgh

10:15 am **Mechanisms and Prognostic Impact of RV Dysfunction in Systemic Sclerosis-PAH**
Paul Hassoun, MD, Johns Hopkins University

10:45 am **Break (15 min)**

11:00 pm **Metabolic and Epigenetic Control of Inflammatory Gene Expression in Pulmonary Hypertension**
Kurt Stenmark, MD, University of Colorado

11:30 am **Apelin system methylation in high-altitude pulmonary edema**
M.A. Qadar Pasha, PhD, Delhi, India

12:00 pm **Lunch and Afternoon Break**

COURSE SCHEDULE

Session V: Novel Therapeutic Strategies for Pulmonary Vascular Disease
Moderator: Cristina Alvira, MD

3:30 pm **Mesenchymal stromal cells and lung repair mechanisms**
Bernard Thebaud, MD, PhD, Ottawa Hospital Research Institute, Canada

4:00 pm **Immunomodulatory effects of MSC Exosomes in lung development and disease**
Stella Kourembanas, MD, Harvard University

4:30 pm **Endothelium-Derived Extracellular Vesicles, MicroRNAs and Pulmonary Hypertension**
Usha Raj, MD, University of Illinois Chicago

5:00 pm **Break (10 min)**

5:10 pm **Tissue engineering in the lung: go or no go?**
Laura E. Niklason, MD, PhD, Yale University

5:40 pm **Abstract Presentation:**
***In Vivo* Generation of Lung Tissue from Embryonic Stem Cells using Blastocyst Complementation**
Bingqiang Wen, PhD, Cincinnati Children's Hospital Medical Center

6:00 pm **Dinner**

7:30 pm **The Estelle Grover Lecture: "Hypoxia Inducible Factors in the developing lung circulation and its response to injury and repair"**
Larissa Shimoda, PhD, MS, Johns Hopkins University

Saturday, September 7, 2019

Session VI: Metabolic Adaptations of the Lung Circulation to Hypoxia
Moderator: David Cornfield MD

7:00 am **Breakfast**

8:00 am **Hypoxia and the lung circulation: An Overview**
Jason X. J. Yuan, MD, PhD, University of California San Diego

8:30 am **How airway hypoxia can provide a window of understanding for lung transplantation**
Mark Nicolls, MD, Stanford University

9:00 am **Hypoxic microenvironments and angiogenesis: Lessons from oncology**
Erinn Rankin, PhD, Stanford University

9:30 am **Break (15min)**

9:45 am **The impact of hypoxia on inflammation, infection and immunity**
Cormac T. Taylor, PhD, University College Dublin, Ireland

10:15 am **Abstract Presentation:**
Evaluation of iPSC-derived Hemangioblasts in Regeneration of Pulmonary Endothelium
Ifeolu Akinnola, BA, University of Minnesota

10:35 am **Abstract Presentation:**
Fatty acid synthesis in neonatal hyperoxia-induced heart disease
Ethan David Cohen, PhD, University of Rochester

COURSE SCHEDULE

10:55 am **Abstract Presentation:**
Single-Cell Transcriptomes Identify Abnormal Endothelial Subpopulation in Pulmonary Arterial Hypertension
Zhiyu Dai, PhD, University of Arizona

12:00 pm **Lunch and afternoon break**

3:30 pm **Bronchial-pulmonary vascular communication during development and in disease**
Csaba Galambos, MD, PhD, University of Colorado

4:00 pm **Transient receptor potential vanilloid 4 channel in permeability edema**
Wolfgang Liedtke, MD, PhD, Duke University

4:30 pm **Break (15 min)**

4:45 pm **Targetable Cellular Signaling Events in the Pathogenesis of Vascular Connective Tissue Disorders**
Harry (Hal) C. Dietz, MD, PhD, Johns Hopkins University

5:20 pm **Infectious Proteinopathy: From an endothelial innate immune surveillance to a transmissible cytotoxicity**
Troy Stevens, PhD, University of South Alabama

6:00 pm **Dinner**

7:00 pm **Evening Poster Session: Chaired by Dr. Mark Gladwin**

Sunday, September 8, 2019

Session VII: Repair and Regeneration: Novel Strategies and Diagnostics
Moderator: Steven Abman, MD

7:00 am **Breakfast**

8:00 am **Molecular determinants of lung vascular zonation for fibrosis-free regeneration**
Shahin Raffi, MD, Cornell University

9:00 am **Towards understanding vascular regeneration**
Luisa Iruela-Arispe, MSc, PhD, University of California Los Angeles

Break (15 min)

9:15 am **Defining the transcriptional and epigenetic basis of endothelial cell heterogeneity**
Joshua Wythe, PhD, Baylor College of Medicine

9:45 am **Epigenetics and the pulmonary vasculature, a therapeutic target?**
William T. Gerthoffer, PhD, Mobile, AL

10:15 am **Closing Remarks**

ABSTRACT PRESENTATIONS

*** Selected for oral presentation**

Protein tyrosine kinases dissociate into BMPR2 supportive and repressive families

Adam M. Andruska[1], Xuefei Tian[1], Rui-Sheng Wang[2], Joseph Loscalzo[2], Edda Spiekerkoetter[1]

[1]Pulmonary and Critical Care Medicine, Stanford University, Stanford CA, [2]Cardiovascular Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston MA.

Introduction: Among the myriad causes of pulmonary arterial hypertension (PAH), impaired BMPR2 signaling is a commonality, though the factors responsible for impairment are elusive. We previously found knockout of the protein Tyrosine Kinase (TK) Lck impaired BMPR2 signaling. TK inhibitors are implicated in the pathogenesis and treatment of PAH but have multiple targets. We aimed to determine how multiple TKs influence BMPR2 signaling.

Hypothesis: TKs segregate into BMPR2 activating and repressive groups with selective TK inhibition projecting a beneficial or deleterious effect in PAH via BMPR2.

Methods: Data from our siRNA screen in C2C12-BRE-luc Id1 reporter cells quantified change in BMPR2 signaling in response to silencing of TKs. BMPR2 modulating TKs were silenced by siRNA in pulmonary artery endothelial cells (PAECs) with qPCR and immunoblotting for BMPR2 signaling. A bioinformatics search using the Broad Institute's ConnectivityMap dataset reverse-identified drugs selectively inhibiting BMPR2-repressive TKs and augmenting BMPR2-activating TKs. Compounds were tested on PAECs with BMPR2 signaling measured by qPCR..

Results: The evolutionarily conserved Src-Family-A TKs repress canonical BMPR2 signaling while the Src- Family B TKs activate BMPR2 signaling per BRE-luc Id1 expression. Knockout of the BMPR2 repressive TK Fyn increased SMAD1 phosphorylation ($p = 0.02$) while knockout of a BMPR2 activating TK LCK reduced Id1 and BMPR2 expression ($p = 0.01$ and 0.02) and increased mesenchymal markers in PAECs. Twenty compounds were identified with the potential to augment BMPR2 signaling, based on their differential PTK targets.

Conclusion: Our data introduces the idea that tyrosine kinases segregate into pro- and anti-BMPR2 groups, which may be relevant for therapeutic development.

Various sulfonamides are protective in lung ischemia-reperfusion injury both by carbonic anhydrase inhibition and other mechanisms

Akshay Kumar and Erik R Swenson

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Background and Objective: TRecent studies from our laboratory have demonstrated the protective action of carbonic anhydrase (CA) inhibition against ischemia-reperfusion (I/R) injury in the heart. However, the mechanisms involved have not been fully elucidated and may involve non-CA inhibition mediated pathways. To examine these for the first time in the lung, we studied effects of acetazolamide (AZ), benzolamide (BZ), and a non-CA inhibiting analog of acetazolamide (n-methyl acetazolamide-NMA) in acute lung I/R injury.

Methods: 30 healthy male rats (300-350 g, 6-8 weeks old) were randomly classified into six groups: 1. Sham (n = 5). 2. I/R injury (n = 5) treated with saline. 3. I/R injury + 30 mg/kg BZ pretreatment (n = 5). 4. I/R injury + 30 mg/kg AZ pretreatment (n = 5) and 5. I/R injury + 30 mg/kg NMA pretreatment (n = 5). Rats in the sham group underwent left thoracotomy without any hilar clamp or treatment. For the IR injury groups, the left main pulmonary artery and bronchus were clamped for 60 minutes and then blood flow was reestablished for 90 minutes, after which measurements were taken and tissue samples obtained.

Results: AZ, NMA, but not BZ, significantly reduced the fall in PaO2/FIO2, a measure of gas exchange efficiency, caused by lung I/R injury. The rise in lung wet to dry weights (reflecting pulmonary edema), and protein extravasation (reflecting capillary permeability) were reduced by all pretreatments. The lungs in pretreatment groups showed less neutrophilic infiltration, alveolar edema and hemorrhage. Lastly, drug pretreatments all reduced HIF-1 activation, suggestive of less tissue hypoxia with I/R injury.

Conclusions: Our data demonstrate that pretreatment with AZ, BZ and NMA all protect against and mitigate the extent of damage with I/R in the lung. The protection appears to involve direct effects of CA inhibition, but in the case of NMA, other actions independent of CA inhibition that may include either anti-oxidative or hypoxia-mediated Ca++ signaling changes, as has been shown in the uninjured hypoxic pulmonary vasculature.

Emergency myelopoiesis contributes to immune cell exhaustion and pulmonary vascular remodeling

Chunhua Fu[1], Yuanqing Lu[1], Mason A. Williams[1], Mark L. Brantly[1], Corey E. Ventetuolo[2], Laurence M. Morel[3], Borna Mehrad[1], Edward W. Scott[4], Andrew J. Bryant[1,4]

[1]Division of Pulmonary, Critical Care, and Sleep Medicine, Department of Medicine, College of Medicine, University of Florida, Gainesville, FL, USA
[2]Division of Pulmonary, Critical Care and Sleep Medicine, Alpert Medical School of Brown University, Providence, Rhode Island, USA
[3]Department of Pathology, Immunology and Laboratory Medicine, University of Florida College of Medicine, University of Florida, Gainesville, FL, USA
[4]Department of Molecular Genetics and Microbiology, University of Florida College of Medicine, University of Florida, Gainesville, Florida, USA..

Abstract: *Background and Purpose* – Pulmonary hypertension (PH) secondary to chronic lung disease (WHO Group 3 PH) is deadly, with lung transplant being the only available long-term treatment option. Myeloid-derived cells are known to affect progression of both pulmonary fibrosi and PH, although the mechanism of action is unknown. Therefore, we investigated the effect of myeloid cell proliferation induced by emergency myelopoiesis on development of PH, and therapy directed against programmed death-ligand 1 (PD-L1), expressed by myeloid cells in prevention of pulmonary vascular remodeling.
Experimental Approach – LysM.Cre-DTR ("mDTR") mice were injected with bleomycin (0.018 U/g, i.p.) while receiving either vehicle or diphtheria toxin (DT; 100 ng, i.p.) to induce severe PH. Approximately four weeks after initiation of bleomycin protocol, right ventricular pressure measurements were performed, and tissue samples collected for histologic assessment. In a separate experiment, DT-treated mice were given anti-PD-L1 (-PD-L1; 500 -g, i.p.) preventive treatment before bleomycin administration.

Key Results: Mice undergoing induction of emergency myelopoiesis displayed more severe PH, right ventricular remodeling, and pulmonary vascular muscularization, compared to controls, without a change in lung fibrosis This worsening of PH was associated with increased pulmonary myeloid-derived suppressor cell (MDSC), particularly polymorphonuclear MDSC (PMN-MDSC). Treatment with PD-L1 normalized pulmonary pressures. PD-L1 expression was likewise found to be elevated on circulating PMN-MDSC from patients with interstitial lung disease and PH.

Conclusions and Implications: PD-L1 is a potentially viable therapeutic target in PH, acting through a signaling axis involving MDSC.

In Vivo Generation of Lung Tissue from Embryonic Stem Cells using Blastocyst Complementation*

*Bingqiang Wen[1], Enhong Li[1], Vladimir Ustiyan[1], Guolun Wang[1], Minzhe Guo2, Cheng-Lun Na2, Gregory T. Kalin1, Yan Xu2, Timothy E. Weaver[2], Tanya V. Kalin[2], Jeffrey A. Whitsett[2], and Vladimir V. Kalinichenko[1,2,]**

[1]Center for Lung Regenerative Medicine and [2]Division of Pulmonary Biology, Perinatal Institute, Cincinnati Children's Research Foundation, Cincinnati, USA

Regeneration or replacement of lung cells or tissues from iPSC or ESC-derived cells represent future therapies for life-threatening pulmonary disorders but are limited by technical challenges to produce highly differentiated cells able to maintain lung function. Functional lung tissue containing airways, alveoli, vasculature and stroma has never been produced from iPSCs or ESCs. We sought to produce all tissue components of the lung from bronchi to alveoli by embryo complementation with ESCs. Blastocyst complementation was used to generate chimeras from normal mouse ESCs and Nkx2-1$^{-/-}$ blastocysts, which lack pulmonary tissues. Pulmonary structures in Nkx2-1$^{-/-}$ chimeras were examined using immunostaining, transmission electronic microscopy, FACS analysis and single cell RNA sequencing. Although peripheral pulmonary tissues are entirely lacking in Nkx2-1 gene deleted embryos, pulmonary lobular structure in Nkx2-1$^{-/-}$ chimeras was restored with airway and alveolar epithelium being derived from the injected ESCs. Multiple respiratory epithelial cell lineages in restored lungs of Nkx2-1$^{-/-}$ chimeras were derived almost entirely from ESCs, whereas endothelial, immune and stromal cells were chimeric. ESC-derived cells from multiple cell lineages were highly differentiated and undistinguishable from endogenous cells based on morphology, ultrastructure, gene expression signatures and cell surface proteins used to identify cells types by FACS.

Conclusions: Entire lung lobes were generated from ESCs by blastocyst complementation. Nkx2-1$^{-/-}$ chimeras can be used as "bioreactors" for *in vivo* differentiation and functional studies of ESC-derived respiratory progenitor cells.

Development of dynamic hydrogel biomaterials for 3D-printing models of human pulmonary vascular disease

Duncan J. Davis-Hall[1], Cassandra L. Petrou[1], Emily E. Thomas[2], and Chelsea M. Magin[1,2]

[1]Department of Bioengineering, University of Colorado, Anschutz Medical Campus and [2]Division of Pulmonary Sciences and Critical Care Medicine, Department of Medicine, University of Colorado, Anschutz Medical Campus

Pulmonary arterial hypertension (PAH) is a severe vascular disease, in which obstructive remodeling of the pulmonary vasculature results in increased pulmonary pressure, progressive right heart failure, and functional decline. Few therapeutic treatments exist to manage it and none directly target the obstructive vascular remodeling that drives pathology. Drug discovery and target validation could be accelerated through improved cell culture techniques that more accurately recapitulate key aspects of human physiology and pathobiology. Specifically it is critical to recreate the 3D structure of the pulmonary vasculature and the time-dependent changes in extracellular matrix mechanical properties that occur during disease progression *in vitro*. We have synthesized and characterized a biodegradable, phototunable poly(ethylene glycol)-based hydrogel that allows investigators to control the mechanical properties of the local microenvironment (i.e., stiffen) on-demand around cells encapsulated within 3D-printed vascular models using focused light, with the goal of emulating PAH initiation and pathogenesis *in vitro*. The best performing material composition demonstrated stiffening from 4.7 ± 0.09 kPa (healthy) to 12.8 ± 0.47 kPa (diseased) and supported cellular adhesion and growth. A two-fold increase in cellular metabolism was measured by a resazurin-based assay in human lung-derived fib oblasts cultured on the new material after 72 hours. It was also possible to 3D-print this material into cylindrical structures with a diameter of 7.5 mm using the freeform reversible embedding of soft hydrogels process. The advanced biomaterial platform implemented here will provide the foundation for models of increasing complexity that reveal novel mechanistic insights into disease prevention and intervention.

Fatty acid synthesis in neonatal hyperoxia-induced heart disease*

Ethan David Cohen, Min Yee and Michael A. O'Reilly

University of Rochester School of Medicine and Dentistry, Department of Pediatrics, Division of Neonatology Rochester NY, 14620-2945

Emerging evidence suggest the use of supplemental oxygen at birth promotes vascular disease and heart failure in adults born preterm through poorly understood mechanisms. We previously found that adult mice exposed to hyperoxia between postnatal days 0-4 develop cardiovascular disease as adults and that this phenotype was preceded by the loss of cardiomyocytes lining the pulmonary vein and left atrium. Here, we show that the expression of enzymes needed for fatty acid synthesis, including Fatty Acid Synthase (*Fasn*), Thyroid Hormone-inducible Hepatic Protein (*Thrsp*), Stearoyl-CoA Desaturase 1 and 2 (Scd1 & 2), and Elongation of long-chain fatty acids family member 4 and 6 (Elovl6), are suppressed in the atria but not ventricles of hyperoxia-exposed neonates. Hyperoxia also suppressed *Fasn, Scd1, and Thrsp* as well as proliferation and survival in HL-1 atrial cardiomyocytes. Pharmacologic inhibition and siRNA knockdown of FASN and SCD1 further reduced the proliferation and survival of HL-1 cells while over-expressing these enzymes preserved growth in hyperoxia. Interestingly, Fasn, Scd1 and other genes needed for fatty acid synthesis remained suppressed in the atria of hyperoxia-exposed mice after recovery in room air. Moreover, aged hyperoxia-exposed mice had reduced cardiac output, stroke volume, ejection fraction and fractional shortening due to poor left ventricular filling suggesting that neonatal hyperoxia causes diastolic heart failure like that observed in adults born preterm. Together, these data suggest that the persistent loss of fatty acid synthesis in pulmonary vein and left atria cardiomyocytes helps initiate the cardiovascular disease and heart failure caused by exposure to hyperoxia in early postnatal life.

Vascular rarefaction and loss of endothelial identity accompanies persistent fibrosis in aged mice

Nunzia Caporarello[1], Aja Aravamudhan[1], Jeffrey A. Meridew[1], Tan Qi[1], Daniel J. Tschumperlin[1], Giovanni Ligresti[2]

[1]Mayo Clinic College of Medicine, Rochester, MN. 2Boston University, Boston, MA

Rationale: Vascular rarefaction has been shown to play an important role in chronic disorders in aged populations, including idiopathic pulmonary fibrosi (IPF). Although altered vascular remodeling is a hallmark of IPF, the contribution of microvascular dysfunction to disease progression remains elusive.

The E-twenty six (ETS)-related gene ERG is a transcription factor that plays a critical role during angiogenesis and vascular stability. The aim of this study was to explore vascular remodeling and ERG function in lung endothelial cells during sustained fibrogenesis

Methods: Vascular density in the lungs of 2 months or 18 months old mice exposed to bleomycin was evaluated by immunohistochemistry. Transcriptional analysis of FACS-sorted lung endothelial cells was assessed by qPCR. *In vitro* loss of function experiments were performed to investigate the role of ERG in endothelial cell function.

Results: We discovered that vascular density was significantl reduced in the lungs of old mice relative to young ones during the resolution phase of bleomycin-induced lung injury. Transcriptional analysis of FACS-sorted lung endothelial cells revealed Erg repression in old mice compared to young ones post-bleomycin along with a reduction of endothelial cell markers and de novo acquisition of mesenchymal markers. *ERG* knockdown in HMEC-L recapitulated the endothelial loss of identity observed in old mice and promoted angiocrine fibroblas activation.

Conclusions: Capillary rarefaction accompanies persistent lung fibrosi in old mice after lung injury. Loss of ERG in lung endothelial cells leads to altered cell identity and may have long-term consequences on capillary stability and angiogenesis. Loss of endothelial cell identity also promotes angiocrine fibroblas activation, potentially contributing to fibrosi progression.

Stabilization of Hypoxia Inducible Factor Improves Lung Structure and Function and Prevents Pulmonary Hypertension in an Antenatal Model of Bronchopulmonary Dysplasia

Gregory Seedorf, Kellen Hirsch, Carly Callahan and Steven Abman

From the Pediatric Heart Lung Center, Departments of Pediatrics, University of Colorado School of Medicine, Aurora CO 80045

Background: Bronchopulmonary dysplasia (BPD), the chronic lung disease of prematurity, is characterized by arrested lung structure and function and high risk for pulmonary hypertension (PH). Animal models suggest that decreased angiogenesis impairs lung growth and causes PH. Hypoxia-inducible factor (HIF) is a key regulator of angiogenesis but whether enhanced HIF signaling prevents BPD is uncertain.

Objective: HThe purpose is to determine if antenatal and postnatal HIF stabilization preserves lung growth and function and prevents PH in an antenatal rat model of chorioamnionitis-induced BPD.

Methods: Endotoxin (ETX, 10ug/sac) was administered to pregnant rats by intra-amniotic (IA) injection at embryonic day 20 (E20; term = E23) and pups were delivered by cesarean-section at E22. Dimethyloxalylglycine (DMOG) or GSK360A was administered to enhance HIF signaling at either E20 (antenatal, 10mgs/sac) or after birth (postnatal, 5 mg/kg IP QOD). At day 14, animals were killed to collect lung tissue to assess alveolarization by radial alveolar counts (RACs); pulmonary vessel density (PVD) by endothelial staining; and cardiac weights to determine right ventricular hypertrophy (RVH; ratio of RV to LV+S weights) as an indicator for PH. Lung protein contents of HIF and vascular endothelial growth factor (VEGF) were determined by western blot. Lung function was determined by Flexivent measurement of compliance and resistance at day 14.

Results: As compared to controls, IA ETX decreased RAC by 42% ($p<0.01$), decreased PVD by 41% ($p<0.01$), increased RVH by 70% ($p<0.01$), increased lung resistance by 46% ($p<0.01$), and decreased lung compliance by 41% ($p<0.01$). Antenatal and postnatal DMOG therapy restored all values to control levels except lung compliance for postnatal therapy. Antenatal GSK360A restored lung structure and function to control values. DMOG increased lung HIF-1 and VEGF protein expression by 4- and 3- fold above values measured after ETX alone ($p<0.01$ for each protein).

Conclusions: We found that antenatal DMOG or GSK360A and postnatal DMOG therapy improves lung structure and function and prevents RVH caused by antenatal ETX exposure. We speculate that the beneficia effects of DMOG and GSK360A therapy are due to HIF stabilization and up-regulation of VEGF expression in the developing lung.

Fatty acid oxidation attenuates hyperoxia-induced endothelial-to-mesenchymal transition

Jiannan Gong[1, 2], Zihang Feng[1], Xuexin Lu[1], Abigail L. Peterson[1], Phyllis Dennery[1,3], and Hongwei Yao[1]

[1]Department of Molecular Biology, Cell Biology & Biochemistry, Brown University; [2]Department of Critical Care and Pulmonary Medicine, Shanxi Medical University Second Hospital, Taiyuan, Shanxi, China; *3*Department of Pediatrics, Warren Alpert Medical School of Brown University

Rationale: Bronchopulmonary dysplasia (BPD) is a chronic lung disease in infants which can lead to pulmonary hypertension (PH). Endothelial-to-mesenchymal transition (EndoMT), a biological process where endothelial cells (ECs) progressively lose their specifi markers and gain mesenchymal phenotype, participates in the pathogenesis of PH. Inhibition of fatty acid oxidation (FAO) augments the EndoMT, which was attenuated by enhancing FAO via overexpression of carnitine palmitoyltransferase 1a (Cpt1a), a rate-limiting step of the carnitine shuttle. However, no information is available on whether hyperoxic exposure as a model of human BPD reduces FAO, resulting in EndoMT. We hypothesized that hyperoxia causes EndoMT by modulating FAO in lung ECs, and pulmonary vascular remodeling in mice.

Methods: Neonatal C57BL/6J mice (<12 h old) were exposed to hyperoxia (>95% O2) for 72 h, which were allowed to recover in air for up to 60 days. MicroCT scan, trichrome, vWF and α-SMA staining were performed in lungs. Lung microvascular ECs were exposed to hyperoxia (95% O2/5% CO2) for 24 h followed by air recovery (O2/rec).

Results: Simplifie vascularization, perivascular fibrosis reduced vessel numbers, increased Fulton index (i.e., the ratio of right ventricular weight to left ventricular plus septal weight) and α-SMA expression in pulmonary vessels were observed in mice exposed to hyperoxia at newborns. Mesenchymal cell markers (i.e., vimentin, CD44, Snail, Slug, Twist1, and α-SMA) were increased, while EC markers (i.e., PECAM-1/CD31 and CD34) were reduced in ECs exposed O2/rec. O2/rec reduced FAO. Cpt1a deletion further aggravated O2/rec-induced CD34 reduction and Slug increase. Enhancing FAO by L-carnitine (0.5 mM, 12h) attenuated O2/rec-induced increase in Slug mRNA in ECs.

Conclusions: Neonatal hyperoxic exposure results in pulmonary vascular and right ventricular remodeling in mice. Hyperoxic exposure causes EndoMT, and enhancing FAO rescues this phenotype. These finding provide novel insights into mechanisms underlying PH in BPD patients.

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Evaluation of iPSC-derived Hemangioblasts in Regeneration of Pulmonary Endothelium*

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Improvements with decellularization and recellularization of decellularized lungs would increase the number of lungs suitable for transplant. One of many hurdles in lung recellularization is the regeneration of a vasculature system with long-term stability. Our goal is to evaluate the potential of hemangioblasts, progenitor cells that can undergo hematopoietic or endothelial differentiation, as a cell source for regeneration of pulmonary endothelium. We hypothesize that the extracellular matrix composition and ultrastructure of a decellularized lung scaffold can be used to differentiate hemangioblasts into endothelial cells and begin recellularization of pulmonary vasculature. A protocol modifie from the Slukvin lab1 was used to create hemangioblasts from iPSCs in the presence of the extracellular protein Tenascin C. Flow cytometry and immunofluorescen microscopy data from differentiated cultures showed increased expression of multiple endothelial markers (CD31, VE-cadherin, and VEGFR2) when hemangioblasts were differentiated on plates coated with Tenascin C compared to vitronectin. To determine the effect of Tenascin C on the recellularization of lung scaffolds, human recombinant Tenascin C was perfused into the vascular lumens of decellularized mouse lungs. Following overnight perfusion in bioreactors, Tenascin C was detected on scaffold cryosections by immunofluorescen staining. Ongoing work is focused on the infusion of rat endothelial progenitor cells (from the D. Alvarez lab) into the vasculature of Tenascin-C-coated scaffolds to demonstrate that Tenascin-C doesn't negatively affect the adherence of endothelial progenitors. This would confir the rationale to seed hemangioblasts in Tenascin-C-coated scaffolds to recreate an endothelium. If so, the functionality and integrity of the resulting endothelium can be carried out. Completion of this work can improve the use of patient derived IPSCs for recellularization of pulmonary vasculature.

Uenishi, Gene et al. "Tenascin C promotes hematoendothelial development and T lymphoid commitment from human pluripotent stem cells in chemically define conditions." Stem cell reports vol. 3,6 (2014)

Integrative Epigenomic and Transcriptomic analysis of Sex-biased Differences in in a Murine Model of Neonatal Hyperoxic Lung Injury

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Background: Male sex is considered an independent predictor for the development of bronchopulmonary dysplasia (BPD). Epigenomic landscape reconfiguratio with profound effects on gene expression is an underappreciated regulatory mechanism and sex-biased differences have not been reported in the murine BPD model.

Objective: Our objective was to test the hypothesis that sex-biased epigenetic modulation of gene expression leads to differential modulation of biological processes in the lung under hyperoxic conditions.

Design/Methods: Neonatal male and female mice (C57BL/6) were exposed to hyperoxia (95% FiO2, PND 1-5: saccular stage) or room air and euthanized on P 7 and 21. Pulmonary gene expression was studied using RNA-seq. Epigenomic landscape was assessed using Chromatin Immunoprecipitation (ChIP-Seq) of the H3K27ac histone modificatio mark, associated with active genes, enhancers, and super-enhancers.

Results: At P7 males showed 153 coordinated genes (12 up, 141 down) whereas females showed 281 genes (40 up, 241 down). However, at P21, males showed only 12 genes (7 up, 5 down) whereas females yielded 138 coordinated genes (47 up, 91 down). Top enriched pathways for males at P7 included angiogenesis, while response to increased oxygen levels was enriched in females. Top enriched pathways for males at P21 included many immune response pathways, while females showed enrichment in vasculature development. H3K27Ac peaks at enhancers and superenhancers also showed significan sex-specifi differences. Interestingly, p21 showed H3K27Ac mediated increase in gene expression in both males and females at P7, but the expression is sustained in females at P21.

Conclusion(s): Dysregulation of transcription via epigenomic landscape alterations may contribute to the sex-specifi differences in hyperoxia-induced inhibition of lung development.

Genomic Analysis of Cellular Signals Driving Neointimal Expansion in an Inflammatory Model of Pulmonary Hypertension

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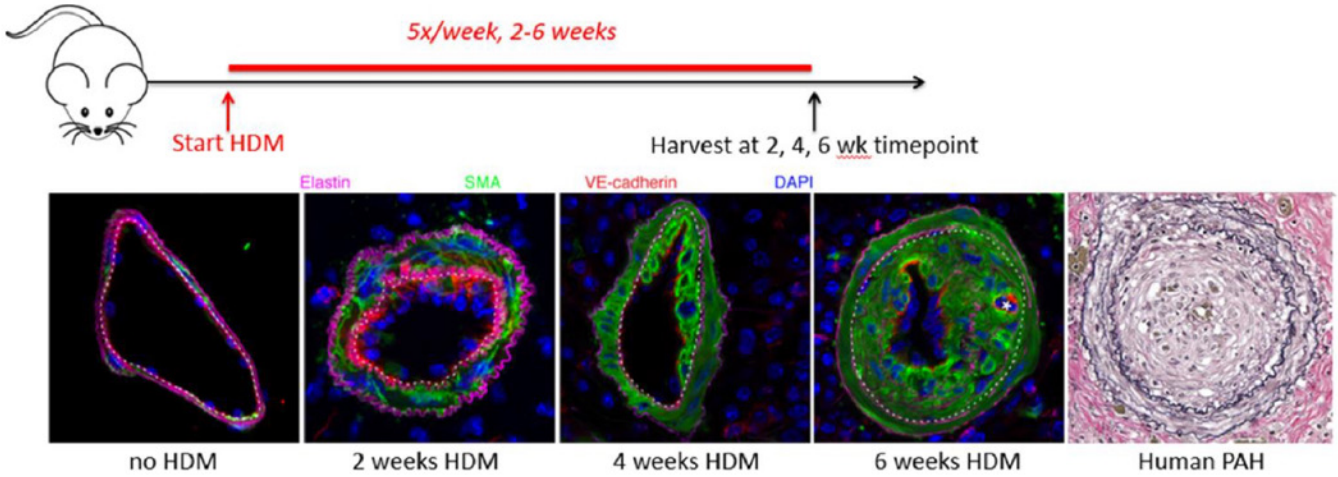
In pulmonary hypertension (PH), the formation of occlusive neointimal lesions is a key driver of elevated pulmonary vascular resistance. Molecular pathways associated with PH have been identified however it remains unclear how these molecules mechanistically contribute to artery remodeling. Improved understanding of the biology of artery remodeling, including the mechanisms that drive neointimal proliferation, could motivate the development of more effective PH therapies. Established mouse models of PH fail to develop significan occlusive neointimal lesions, impeding our ability to study the cellular mechanisms driving lesion formation. To address this fundamental limitation, we developed an inflammation-base mouse model using chronic intranasal house dust mite exposure that shares many features of human PH including progressive vasculopathy with extensive neointimal hyperplasia (Figure 1). Using CreER based lineage cell tracing, we have demonstrated that neointima is derived from smooth muscle cells of the artery wall without contribution from vascular endothelial cells. The morphology and behavior of the neointima is distinct from medial smooth muscle cells, and shows striking similarities to that seen in humans. Proliferating neointimal cells are strongly biased to being located immediately adjacent to the endothelium, suggesting interactions between neointima and endothelium promote neointimal proliferation. Using single cell RNA-seq datasets generated from neointima, smooth muscle, and endothelial cells from both healthy and PH mice we are identifying patterns of transcription that defin neointimal cells. Using these datasets we are mapping signaling interactions that are candidate drivers of neointimal proliferation. Top candidates will be functionally tested in vivo using both genetics and pharmacology for a role in halting the development of occlusive vascular lesions or potentially reverse established disease.



Insulin Like Growth Factor Binding Protein 2 is a novel outcome marker for Pediatric Pulmonary Hypertension

Megan Griffiths, MD, Jun Yang, PhD, Melanie Nies, MD, Dhananjay Vaidya, MD PhD, Stephanie Brandal, MS, Monica Williams, MD, Elizabeth C. Matsui, MD MHS,,Rachel Damico, MD PhD, Dunbar Ivy, MD, Eric D. Austin, MD, William Nichols, PhD, Michael Pauciulo, BS, Katie Lutz, BS, Erika B. Rosenzweig, MD, Russel Hirsch, MD, Delphine Yung, MD, Allen D. Everett, MD

Rationale: Pediatric pulmonary arterial hypertension (WSPH Group 1 PAH) is a progressive disease characterized by elevated pulmonary arterial pressures. An agnostic plasma proteomics discovery approach identifie insulin like growth factor binding protein 2 (IGFBP2) as a possible biomarker of PAH. Insulin like growth factors (IGFs) promote endothelial growth and production of nitric oxide. IGFs, and their binding proteins (IGFBPs) impact cardiopulmonary function and may influenc the pathobiology of PAH.

Objective: Determine the diagnostic and prognostic value of IGF1, IGF2 and IGFBP2 in pediatric PAH.

Methods: Using an enzyme-linked immunosorbent assay, we evaluated serum IGF1, IGF2, and IGFBP2 in pediatric PAH subjects (0-21 years) from the NHLBI PAH Biobank (N=175), matched healthy controls (N=75, age 0-21), and a cohort of lung disease (asthmatic) controls (N=46, age 0-21) enrolled in the CATCH study. Serum IGF1, IGF2 and IGFBP2 were analyzed with demographic, clinical and hemodynamic variables for PAH diagnosis and severity.

Results: Serum IGFBP2 concentrations were elevated in PAH compared to both cohorts of controls (264ng/mL, 208ng/mL, 99ng/mL respectively; p<0.00001). IGF1 negatively correlated with pulmonary vascular resistance index, PVRi, (R=-0.534, P=0.003), while IGFBP2 positively correlated with PVRi (R=0.669, P<0.01). IGFBP2 negatively correlated with cardiac output (r= -0.481, p=0.008) and 6-minute walk distance (r=-0.706, p=0.003). Higher median IGFBP2 levels were associated with use of IV/subcutaneous prostacyclin (IV/SQ PCA; median IGFBP2 363ng/mL, IQR 247-508, p<0.0001). In logistic regression analysis adjusted for age and sex, higher IGFBP2 had an adjusted odds ratio of 6.78 for mortality (p=0.016).

Conclusion: Circulating IGFBP2 is a novel prognostic marker for pediatric PAH with ability to distinguish more severe disease, worse functional status, need for chronic infusion therapy, and survival. The reciprocal hemodynamic association of IGF1 and IGFBP2 suggest dysregulation of the IGF axis. IGF axis dysregulation may be an important mechanistic target in pediatric pulmonary arterial hypertension.

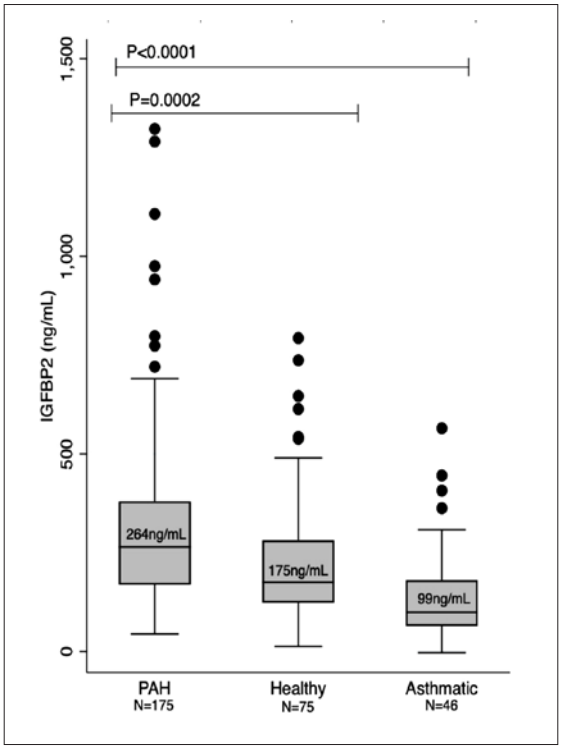


Figure 1. IGFBP2 concentration (ng/mL) in PAH versus healthy controls versus asthmatic

Table 1: Adjusted Linear Regression of IGFBP2, IGF1, and IGF2 against functional outcomes (N=29, subjects with RHC within 6 months of enrollment; adjusted for age and gender)

	IGF1 Coefficient (95% CI)	Semipartial Correlation	P-Value	IGF2 Coefficient (95% CI)	Semipartial Correlation	P-Value	IGFBP2 Coefficient (95% CI)	Semipartial Correlation	P-Value
Six Minute Walk Distance (m)	**130 (28.4, 231.7)**	**0.585**	**0.017***	-3.4 (-136, 129)	-0.015	0.96	**-140.4 (-235, -45)**	**-0.633**	**0.008***
PVR (WU)	**-4.76 (-8.8, -0.7)**	**-0.419**	**0.02***	-0.32 (-5.3, 4.7)	-0.025	0.89	**4.89 (0.32, 9.4)**	**0.387**	**0.037***
PVRi (WUi)	-0.79 (-7.1, 5.5)	-0.035	0.79	-3.5 (-9.9, 2.9)	-0.149	0.27	5.0 (-1.26, 11.3)	0.211	0.112
mPAP (mmHg)	**-10.5 (-19.4, -1.6)**	**-0.429**	**0.02***	-1.3 (-11.5, 8.9)	-0.05	0.8	8.6 (-1.78, 19)	0.316	0.1
mRAP (mmHg)	**-1.7 (-3.15, -0.17)**	**-0.409**	**0.03***	-061 (-2.3, 1.1)	-0.146	0.46	0.88 (-0.9, 2.67)	0.196	0.32
mPCWP (mmHg)	**-1.49 (-2.7, -0.27)**	**-0.449**	**0.019***	-1.22 (-2.5, 0.10)	-0.355	0.07	0.56 (-0.94, 2.07)	0.154	0.45
Cardiac Output (L/min)	0.37 (-0.35, 1.1)	0.175	0.3	-0.33 (-1.1, 0.42)	-0.154	0.37	-0.73 (-1.5, 0.032)	-0.312	0.06

Adjusted Logistic Regression of IGFBP2, IGF1, and IGF2 against functional outcomes (N=175, all enrollees)

	IGF1 Coefficient (95% CI)	P-Value	Odds Ratio	IGF2 Coefficient (95% CI)	Odds Ratio	P-Value	IGFBP2 Coefficient (95% CI)	Odds Ratio	P-Value
Dyspnea	0.62 (-0.19, 1.43)	0.14	0.36	-0.31 (-0.99, 0.37)	0.73	0.36	0.31 (-0.39, 1.0)	1.36	0.39
Mortality	-1.0 (0.064, 1.19)	0.116	1.85	-0.41 (-1.7, 0.9)	0.67	0.54	**1.19 (0.36, 3.5)**	**6.78**	**0.016***

Altered pulmonary vasculature development, reticulocyte maturation and increased hemolysis in PH model of severe mitochondrial dysfunction

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Humanized point mutation (G206C) in iron-sulfur cluster scaffold protein NFU1 induces a severe mitochondrial dysfunction and spontaneous pulmonary hypertensive (PH) phenotype in rats. The same mutation also associates with severe pulmonary vascular remodeling. In this study, we evaluated whether NFU1G206C rats have an altered post-natal development of pulmonary vasculature that could contribute to the phenotype.

It has been reported that the complexity of pulmonary vasculature in rats progressively increases from neonatal age to adulthood. We found that these changes correspond with a decrease in the initially elevated erythropoiesis and the amount of circulating reticulocytes (RTC). In contrast, NFU1 mutant rats showed severe vascular-obliterative disease and reduced number of small vessels and branches that stay preserved in adulthood. The impairment in pulmonary vascular morphology associated with a continuously elevated RTC count.

The process of RTC maturation is known to be mitochondrial-dependent; thus, mitochondrial dysfunction mediated by NFU1 mutation could impair the maturation of RTC and increase the risk of their rupture. Indeed, NFU1 mutant rats show an accumulation

of immature RTC and a significantl elevated level of circulating free hemoglobin as a marker of hemolysis. The progressive nature of these changes correlated with the progression of PH.

The *ex vivo* maturation of RTC isolated from NFU1^{G206C} rats was restored to normal by supplementation of lipoic acid, an important mitochondrial cofactor, which synthesis was impaired by NFU1 mutation. Importantly, we found that 7 out of 10 patients with idiopathic pulmonary arterial hypertension (IPAH) have lipoic acid insufficiency Taken together, these results suggest that non-recognized alteration in lipoic acid synthesis could contribute to impaired RTC maturation, elevated hemolysis, and IPAH progression.

A single mutation in NFU1 gene metabolically reprograms pulmonary artery smooth muscle cells

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NFU1 is an iron-sulfur (Fe-S) scaffold protein, involved in the Fe-S assembly and transfer to a range of mitochondrial metalloproteins. Patients with the NFU1^{G208C} mutation develop pulmonary arterial hypertension (PAH) with 70% penetrance. Rats with NFU1^{G206C} homozygous mutation demonstrated the PAH phenotype showing increased pulmonary vasculature remodeling, right ventricular (RV) hypertrophy, and RV pressure. We analyzed the mitochondrial proteome in NFU1^{G206C} homozygous mutant rats and discovered the phenotypic changes in pulmonary arterial smooth muscle cells (PASMC) that were dictated by the mitochondrial proteome alterations. Quantitative analysis of the mito-proteome showed significan changes in 208 proteins involved in various metabolic and antioxidant functions in response to the NFU1 mutation. Our data indicates that the NFU1^{G206C} homozygous mutant rats have decreased expression of complex I and II, which are known to depend on iron-sulfur clusters, and increased expression of complexes III to V, accompanied with a mitochondrial dysfunction, amplifie glycolysis and anabolism in PASMC. Our data indicate involvement of NFU1 mutation in a dysregulated antioxidant system in the mitochondria leading to increased levels of reactive oxygen species (ROS). Due to alterations in apoptosis regulating proteins, these cells exhibited high proliferation rates and resistance to apoptosis as compared with the wild type (WT). Finally, the mito-proteome showed significan disturbances in proteins regulating fatty acid (FA) metabolism and our functional characterization showed increased FA oxidation in the PASMC. In conclusion, we found that the NFU1^{G206C} mutation induces a metabolic reprogramming in the PASMC by glycolytic switch, reduced glucose oxidation and increased fatty acid oxidation, and amplifie ROS production resulting in a hyper-proliferative and apoptosis resistant phenotype, and presents a novel cellular model to study PAH.

Antioxidant conjugated peptide attenuated metabolic reprogramming in pulmonary hypertension

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Pulmonary arterial hypertension (PAH) is a severe cardiopulmonary disorder instigated by pulmonary vasculature proliferation. Activation of Akt signaling was previously reported to promote vascular remodeling. It was found that the irreversible nitration of Y350 residue in Akt results in its activation. Our data indicate that Akt nitration is increased in patients with PAH and SU5416/Hypoxia animal model. This study investigated whether prevention of Akt nitration in PAH by the targeted antioxidant peptide could reverse vascular remodeling and metabolic reprogramming.

The designed peptide, aimed to prevent nitration of Akt, had an affinit to Akt with an antioxidant moiety – nitroxide, conjugated to the peptide. Treatment of SU/Hypoxia model with the antioxidant peptide for two-weeks significantl reduced nitration of Akt in lungs, attenuated right ventricle (RV) hypertrophy and reduced RV systolic pressure. Metabolically, SU/Hypoxia model characterized with increased glycolysis, anaplerosis and decreased glucose oxidation. Akt nitration was found to induce glycolysis by activation of glucose transporter GLUT4, hexokinase-1, and glyceraldehyde 3-phosphate dehydrogenase enzymes and decrease glucose 6-phosphate dehydrogenase and increased glycogen synthase kinase 3β. This enhances the flu of glucose via glycolysis in PAH. The increased glycolytic rate upregulates anaplerosis via activation of pyruvate carboxylase in SU/Hypoxia rats. The Akt targeted antioxidant peptide resolved glycolytic switch, activated pentose phosphate, and glycogenesis pathways. Prevention of Akt nitration significantl controlled pyruvate in oxidative phosphorylation by decreased lactate and increased pyruvate dehydrogenases activities. Finally, histopathological studies showed significantl reduced pulmonary vascular proliferation.

Conclusion: Based on our current observation, we infer that preventing Akt nitration using an Akt-targeted antioxidant peptide could be a useful treatment option for controlling vascular proliferation in PAH.

Mitochondrial Calcium Buffering Determines Lung Endothelial Barrier Properties

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Rationale: Mitochondrial Ca2+ buffering (MCB) is a protective response in which mitochondria take up Ca2+ to protect against cell damaging effects of increases in cytosolic Ca2+ (cCa2+). In a mouse model of acid aspiration-induced acute lung injury (AALI), we tested the hypothesis that MCB failure underlies endothelial barrier loss due to alveolar HCl instillation.

Methods: We viewed live alveoli of isolated blood-perfused mouse lungs by confocal microscopy. Then, we microinstilled HCl (pH 1.1) by alveolar micropuncture. By intravascular infusion we gave the potentiometric mitochondrial dye TMRE, the cCa2+ detector fluo-4 and the capillary hyperpermeability marker, fluorescen 70-kD dextran (FD70).

Results: Confocal imaging indicated that at baseline, the fluorescenc intensities of endothelial TMRE and fluo- were steady, and alveolar FD70 fluorescenc was absent, indicating that endothelial mitochondrial potential, cCa2+, and barrier were stable. However, within 10 min after alveolar acid instillation TMRE fluorescenc decreased, fluo- fluorescenc increased, and FD70 leaked into alveoli (n=4, $P<0.05$), indicating acid instillation induced mitochondrial depolarization, cCa2+ increase, and barrier deterioration. Pretreating microvessels with siRNA to knockdown endothelial uncoupling protein-2 (UCP2), blocked all three responses ($P<0.05$).

Conclusions: We interpret that alveolar instillation of HCl activated endothelial UCP2, causing rapid loss of endothelial MCB, hence increase of endothelial cCa2+. Since UCP2 is a proton channel in the mitochondrial inner membrane, proton shunting across activated UCP2 caused the depolarization, abrogating MCB. The UCP2 activation caused endothelial barrier loss and alveolar edema. These finding are novel evidence that acid contact with the alveolar epithelium causes rapid, UCP2-dependent loss of endothelial barrier properties. Therapeutic targeting of UCP2 might constitute new therapy for AALI. (HL105323, Stony Wold-Herbert Fund).

Loss of AC10 impairs neutrophil recruitment into the lung following Pseudomonas aeruginosa infection

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Pseudomonas aeruginosa is a gram-negative, opportunistic bacteria and a common cause of nosocomial pneumonia in critically ill patients that can progress to acute respiratory distress syndrome (ARDS). These patients have impaired gas exchange leading to hypoxia, altered blood pH and elevated bicarbonate. Bicarbonate, delivered into the cell via sodium bicarbonate cotransporters (NBCs) activates adenylyl cyclase 10 (AC10) to increase intracellular cAMP. Indeed, bicarbonate activation of AC10 is required for LPS-induced lung endothelial permeability. Neutrophil recruitment into the airspaces plays a central role in the progression of ARDS. Recently, AC10 has been implicated in transendothelial migration of neutrophils into the underlying tissue. We sought to determine whether AC10 is necessary for neutrophil recruitment into the airspaces following *P. aeruginosa* intratracheal inoculation. Increasing doses of bacteria were introduced directly into the lung of wild type and AC10 and NBCn2 knockout mice. Bronchoalveolar lavage flui (BALF) was recovered 24 hours later and examined for recruitment of polymorphonuclear cells (PMNs) into the airspaces. The number of PMNs recruited into the airspace following *Pseudomonas* inoculation increased with bacterial number and reached a maximum at a dose of 1E5 bacteria after which 100% of cells in the BALF were PMNs. Thus, doses upto 1E5 bacteria were used to examine the role of AC10 and NBCn2 in PMN recruitment into the airspace between wild type and knockout animals. Our data reveal a decreased number of PMNs in the BALF of both AC10 and NBCn2 knockout female mice; however, there no difference in the number of PMNs recruited into the airspace was detected between male wild type and knockout animals. Thus, female mice utilize AC10 and NBCn2 to facilitate neutrophil recruitment into the airspace following a model of *P. aeruginosa pneumonia*.

Gestational high altitude hypoxia and phenotypic transformation of pulmonary arteries from fetal sheep

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University of New Mexico; California Baptist University; Loma Linda University School of Medicine; West Coast Metabolomics Center; University of California at Davis; United States Department of Agriculture; Western Human Nutrition Research Center; California Polytechnic State University at San Luis Obispo

Gestational high altitude hypoxia increases the risk of myriad diseases in human infants including pulmonary hypertension of the newborn. Fetal sheep are susceptible to long-term intrauterine hypoxia, with the lung structure and function exhibiting characteristics associated with the development of pulmonary hypertension. There is a thickening of the pulmonary arterial medial wall, dysregulation of arterial reactivity, and reduced right ventricular output. To further explore the mechanisms associated with hypoxia induced aberrations in the fetal sheep lung, we examined the premise that proteomic and metabolomic changes are associated with intrauterine hypoxia.To address this hypothesis, we performed proteomic and metabolomic analysis on pulmonary arteries isolated from near term fetuses that were exposed to intrauterine high altitude hypoxia for the latter 100+ days of gestation or that gestated near sea level.There is a glycolytic shift and an increase in pentose phosphate metabolism, illustrative of increases in nucleotide synthesis.There is also a reduction in smooth muscle myosin, fibronectin and collagen and upregulation of proteins involved in nucleotide synthesis. While our observed changes are consistent with a hypoxia-induced phenotypic shift towards smooth muscle cells being more synthetic, the proteomic and metabolomic biomarkers also suggest hypoxia is causing other cells to mature. Such finding provide insight into the mechanisms related to gestational hypoxia mediated pulmonary hypertension, however further research is needed to understand the underpinnings associated with the observed phenotypic transformations.

Single Cell Sequencing and Lineage Tracing of Pulmonary Vasculature Development

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Boston University, Center for Regenerative Medicine (CreM)

Rational: Temporospatial development of the pulmonary vasculature is poorly understood. Comprehending the cellular interactions that specify the pulmonary vasculature could lead to new therapies for conditions such as pulmonary hypertension. We aim to characterize vascular precursors in murine embryonic lungs at different developmental stages and the signals that drive vascular maturation.

Methods: Cdh5-CreER mice were crossed with ROSA-TdTm reporters and injected with tamoxifen at E8.5, to mark the progeny of early vascular progenitor cells for genetic lineage tracing. Lungs were harvested at E14.5 to analyze the lineage traced progeny using immunofluorescenc (IF) staining, FACS analysis and single cell RNA sequencing. Our objective is to determine when and how these progenitor cells give rise to the cell types of the mature pulmonary vasculature.

Results: When tamoxifen was given to induce recombination at E8.5 and lungs were analyzed at E14.5, we detected TdTm+ venous, arterial and capillary endothelial cells, suggesting there is a multipotent vascular precursor population as early as E8.5. A total of 1104 individual TdTm+ lineage traced cells were sequenced (using the 10X platform), yielding a median 2935 genes per cell.

Conclusion: Preliminary analysis of the single cell RNA sequencing data has identifie a subpopulation of endothelial cells with a different genetic profile confirmin a multipotent vascular precursor that gives rise to different subpopulations of endothelial cells. We will mine the RNA sequencing data to identify molecular pathways that direct the development and maturation of the pulmonary vasculature.

Putative Role of Endothelial Cell-derived Cav-1+ Extracellular Vesicles in Schistosomiasis-associated PAH

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Introduction: Microvascular muscularization and formation of plexiform lesions, hallmark features of idiopathic PAH, are also observed in Schistosomiasis-associated PAH suggesting their etiology in both groups may share mechanistic features. *Schistosoma mansoni* infection has been linked to EC dysfunction[1,2] and vasoconstriction[3] and although endothelial cell (EC) dysfunction is thought to play a primary role in the etiology of PAH[4,5], *the mechanism is still unknown*.

Objective: We sought to evaluate if inflammator response-associated to *S. mansoni* egg antigens alter EC Caveolin-1 (Cav-1) and BMPRII expression via shedding of extracellular vesicles (EV) and whether this contributes to PAH.

Results/Discussion: EC-Cav-1 depletion in IPAH patients and rodent models is mediated in part by shedding of EV, which contributes to BMPRII depletion and recruitment of TGF-β-producing macrophages[6]. In the lungs of *S. mansoni*-infected mice, we observed a significan loss of Cav-1 expression only in the egg-dependent granuloma area. *In vitro*, *S. mansoni* egg antigen Sm-p40 (1 µg/mL; 48 hs) did not alter Cav-1 expression in human pulmonary artery EC. However, exposure of microvascular EC to Sm-p40 for 1 hr increased Cav-1 Tyr14 phosphorylation (228.2 +/- 38.28% of control; $p<0.05$; $n = 4$) and co-incubation (18 hr) with either IL-6 or TNF-α induced shedding of EV.

Conclusion: This work is uncovering the role and potential clinical relevance of EC-Cav-1 depletion via EV shedding as a biomarker of severe pulmonary vascular injury and remodeling during PAH.

Funding: Postdoctoral fellowships from CNPq/CsF and an award from the American Heart Association and Circle of Service Foundation.

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Nanoparticle Delivery of Proangiogenic Transcription Factors into Endothelial Cells Inhibits Alveolar Simplification Caused by Neonatal Hyperoxia

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Advances in neonatal critical care have greatly improved the survival of preterm infants but the long-term complications of prematurity, including Bronchopulmonary dysplasia (BPD), cause mortality and morbidity later in life. While Vascular Endothelial Growth Factor (VEGF) improves lung structure and function in rodent BPD models, severe side effects of VEGF therapy prevent its use in BPD patients. In the present study, we used mouse BPD model to test whether nanoparticle delivery of proangiogenic transcription factors FOXM1 or FOXF1, both downstream targets of VEGF, can improve lung structure and function after neonatal hyperoxic injury. Novel formulation of nanoparticles (PEI_{600}-MA_5/PEG-OA/Cho) has been developed to deliver non-integrating expression plasmids containing *FOXM1* or *FOXF1* cDNAs into pulmonary microvascular endothelial cells *in vivo*. The nanoparticles targeted 80-90% of endothelial cells without targeting other cell types in the lung. A single dose of either FOXM1 or FOXF1 was proangiogenic and bypassed side effects of VEGF, increasing lung septation after neonatal hyperoxic injury. FOXM1 or FOXF1 did not protect endothelial cells from apoptosis caused by hyperoxia but increased endothelial proliferation after the injury. FOXM1 and FOXF1 improved elastin fibe organization, decreased alveolar simplificatio and preserved lung function in mice reaching adulthood.

Conclusions: Nanoparticle delivery of FOXM1 or FOXF1 stimulates lung angiogenesis and alveolarization during recovery from neonatal hyperoxic injury. Gene therapy using pro-angiogenic transcription factors has promise to prevent BPD associated with pulmonary vascular disease.

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Pulmonary hypertension (PH) involves remodeling of pulmonary arteries, featuring proliferation, migration and reduced apoptosis of pulmonary arterial smooth muscle cells (PASMCs). The mechanisms controlling PASMC homeostasis are incompletely understood. Our laboratory identifie AQP1, a water transporter, as a novel regulator of PASMC function. We have reported that hypoxia upregulated AQP1 in rat PASMCs from distal pulmonary arteries via a Ca2+-dependent mechanism. We now show that AQP1 protein is upregulated by HIF-1 and HIF-2, not as a direct transcriptional target, but secondary to HIF-dependent increases in intracellular Ca2+. Elevating AQP1 levels by hypoxia or an adenovirus containing wild-type AQP1 (AdAQP1) increased PASMC proliferation and migration, whereas a mutant form of AQP1 (AdAQP1CT) lacking the C-terminal tail but with normal water transport did not. In PASMCs, infection with AdAQP1, but not AdAQP1CT, increased β-catenin protein levels and expression of β-catenin target genes. Silencing β-catenin prevented the effect of AdAQP1 on PASMC migration and proliferation. To determine whether AQP1 regulates PASMC survival and/or susceptibility to apoptosis, we used PASMCs from the SU5416-hypoxia (SuHx) rat model of PH. SuHx PASMCs exhibited increased AQP1 protein and were resistant to apoptosis induced by H2O2. Depleting AQP1 with siRNA induced apoptosis at baseline in both control and SuHx rat PASMCs, measured by Hoechst staining, cleaved caspase-3 and caspase-3 activity, and sensitized SuHx PASMCs to apoptotic stimuli. Our results indicate that AQP1 is a critical regulator of PASMC function, controlling β-catenin levels to regulate migration and proliferation and regulating activation of caspase 3, the executioner of apoptosis. These finding suggest targeting AQP1 might provide an attractive therapeutic option to halt or reverse vascular remodeling in PH.

Pulmonary microvascular engineering using purified pulmonary microvascular endothelial cells

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Background: In whole lung engineering, functionally endothelialized pulmonary microvasculature is critical for establishing a fluid-tigh barrier in alveolar compartment, so that oxygen and carbon dioxide can be exchanged in the organ. However, derivation of a purifie and proliferative microvascular endothelium has been hampered due to an incomplete understanding of endothelial heterogeneity. Here, we isolated a purifie lymphatic low pulmonary microvascular endothelium (lymphlow PMEC) that is molecularly homogenous and capable of repopulation of decellularized lung scaffolds.

Methods: By using Prox1-egfp, and Lyve-1 as markers, lymph^{low} PMECs were isolated from regions of low lymphatic in rat lungs. Molecular profile of lymph^{low} PMECs were analyzed by single cell RNA sequencing (scRNAseq) as compared to native counterparts. These cells were then used to culture in decellularized lung scaffolds.

Results: Lymph^{low} PMECs were morphologically homogeneous as early as P1 and capable of rapid expansion. ScRNAseq data revealed that P1 lymphlow PMECs retained heterogeneous population of endothelium including microvascular, arterial, and lymphatic endothelium. Intriguingly, P4 lymph^{low} PMECs contained molecularly homogenous cell population with neither lymphatic, nor arterial endothelium present. Pair-wise comparison revealed that both early and late passaged lymphlow PMECs display more similar molecular profile to endothelium in medium-sized vessels rather than that in capillary and big vessels in native lungs. After culturing in decellularized lung scaffolds, lymph^{low} PMECs can well repopulate (>50% coverage relative to native) and the microvascular markers such as Prx, Apln, and Kdr were increased.

Conclusions: these results not only provide unprecedented molecular profile of heterogeneous pulmonary endothelium in native tissues and in in vitro culture, but also provide a purifie cell candidate for pulmonary microvascular engineering.

Single-Cell Transcriptomes Identify Abnormal Endothelial Subpopulation in Pulmonary Arterial Hypertension*

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Introduction: Pulmonary arterial hypertension (PAH) is a disaster disease characterized by obliterative vascular remodeling and persistent increase of vascular resistance, leading to right heart failure and premature death. Understanding the cellular and molecular mechanisms will help develop novel therapeutic approaches for PAH patients.

Hypothesis: We hypothesis that endothelial plasticity or distinct cell populations are critical for obstructive vascular remodeling in the pathogenesis of PAH.

Methods: Here we applied single-cell RNA sequencing (ScRNA-seq) to profil the pulmonary cells in a severe mouse model (*Tie2Cre*-mediated deletion of *Egln1* [encoding Prolyl-4 Hydroxylase 2 (PHD2)], designated *Egln1*^{Tie2Cre} mice) of PAH.

Results: ScRNA-seq revealed 20 discrete cell populations from pooled mouse lung single cells from WT and *Egln1*^{Tie2Cre} mice. We identifie fiv distinct EC subpopulations in both WT and *Egln1*^{Tie2Cre} mice, which expressed classical EC markers *Emcn, Pecam1* and *Cdh5*. Unexpectedly, there were markedly difference in second abundant EC Cluster (EC2) between WT and Egln1Tie2Cre lung. The number of Cluster (EC2) was markedly increased in CKO lung compared with from WT lung. EC2 cluster (mainly from *Egln1*^{Tie2Cre} lung) was characterized by little expression of *Cldn5, Tmem100, Tspan7, Calcrl* and *Foxf1*. Analysis of genes related to the pathogenesis of PH showed that angiocrine factor genes *Pdgfb, Cxcl12, Mif* and *Edn1* are significantl increased in all EC subpopulations from *Egln1*^{Tie2Cre} mice compared to WT mice. We also analyzed genes which were found to be mutated in human PAH patients and found that some of these genes (*Sox17, Atp13a3* and *Smad4*) were ubiquitously upregulated in all EC subpopulations, some of these genes were selectively downregulated or upregulated in specifi EC subpopulation(s) [down: *Bmpr2, Acvrl1, Aqp1, Ptgis, Cav1; Up: Eif2ak4* and *Smad1*] in *Egln1*^{Tie2Cre} mice compared to that from WT mice.

Conclusions: ScRNA-seq analysis identifie unique endothelial population only highly enriched in the lung of severe PAH mice.

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