GROVER CONFERENCE

2017

September 6-10, 2017

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THE PROGRAM

About the Program

Since its inauguration in 1984, the 2017 Grover Conference will be the 18th 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

This four-day conference includes lectures, discussions, and poster presentations to develop a better understanding of state-of-the-art bench research with clinical management and drug development strategies for pulmonary hypertension.

Learning Objectives

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

1. Understand phenotypic diversity and role of vascular endothelium in initiation of lung injury and repair
2. Understand a multi-faceted role of pulmonary circulation and specifically, vascular endothelium in pathogenesis and modulation of lung diseases (ALI/ARDS, PH, COPD, ILD and CF)
3. Recognize the importance of the improvement of vascular function and endothelial health in various lung diseases as part of clinical practice.
4. Formulate future endothelial-centric research directions to treat lung disease.

Who Should Attend

Physician scientists, basic research and translation research scientists, clinicians, nurses, research and clinical fellows, graduate students.

PROGRAM COMMITTEE

Konstantin G. Birukov, PhD, MD, Chair, Organizer
Troy Stevens, PhD
Wolfgang M. Kuebler, MD

SPEAKERS AND SESSION CHAIRS

Jahar Bhattacharya, MD, Columbia University Medical Center, New York, NY

Konstantin G. Birukov, PhD, MD, University of Maryland School of Medicine, Baltimore, MD

Anna Birukova, MD, University of Maryland School of Medicine, Baltimore, MD

Sebastien Bonnet, PhD, MSc, Research Center of the Institute of Cardiology & Pulmonology of Quebec, Quebec City, QC

Stephen Y. Chan, MD, PhD, University of Pittsburgh, Pittsburgh, PA

Mark de Caestecker, MBBS, PhD, Vanderbilt University Medical Center, Nashville, TN

David Cornfield, MD, Stanford University, Stanford, CA

Vinicio De Jesus Perez, MD, Stanford University, Stanford, CA

Steven M. Dudek, MD, University of Illinois at Chicago, Chicago, IL

Serpil C. Erzurum, MD, Cleveland Clinic Foundation, Cleveland, OH

Christophe Guignabert, PhD, French Institute of Health and Medical Research, Paris, France

Wolfgang M. Kuebler, MD, Charité – Universitätsmedizin Berlin, Berline, Germany

Mandy R. MacLean, PhD, University of Glasgow, Glasgow, Scotland

Susan M. Majka, PhD, Vanderbilt University Medical Center, Nashville, TN

Asrar B. Malik, PhD, University of Illinois at Chicago, Chicago, IL

Michael A. Matthay, MD, University of California at San Francisco, San Franciso, CA

Vladimir R. Muzykantov, MD, PhD, University of Pennsylvania, Philadelphia, PA

Caroline A. Owen, MD, PhD, Brigham and Women's Hospital, Boston, MA

Irina Petrache, MD, National Jewish Health, Denver, CO

Sharon I. S. Rounds, MD, Warren Alpert Medical School of Brown University, Providence, RI

Eric P. Schmidt, MD, University of Colorado School of Medicine, Aurora, CO

Kurt R. Stenmark, MD, University of Colorado Denver, Aurora, CO

Troy Stevens, PhD, University of South Alabama of Medicine, Mobile, AL

Duncan J. Stewart, MD, Ottowa Health Research Institute, Ottawa, ON

Mary I. Townsley, PhD, University of South Alabama of Medicine, Mobile, AL

Rubin M. Tuder, MD, University of Colorado School of Medicine, Aurora, CO

Stefan Uhlig, PhD, Institute of Pharmacology & Toxicology, Aachen, Germany

Norbert F. Voelkel, MD, VU University Medical Center, El Prado, NM

E. Kenneth Weir, MD, University of Minnesota Medical School, Wayzata, MN

Norbert Weissmann, PhD, Justus Liebig University of Giessen, Giessen, Germany

R. James White, MD, PhD, University of Rochester, Rochester, NY

Mervin C. Yoder, MD, Indiana University School of Medicine, Indianapolis, IN

2017 GROVER CONFERENCE
on the Endothelium at the frontline of Vascular Pathobiology and Therapeutic Targeting in Lung Vascular Disease

COURSE SCHEDULE

Wednesday, September 6, 2017

12:00 pm **Arrivals**
6:00 pm **Welcome Reception and Dinner**

Thursday, September 7, 2017

A. Endothelial Pathobiology and Targeting in ARDS and Inflvoammatory Lung Disease
Session I: Molecular Basis of Endothelial Dysfunction and Therapeutic Targeting in ARDS
Moderator: Asrar Malik, PhD

7:00-8:00 am Breakfast

8:05 am Welcome and Introduction
Konstantin Birukov, PhD, MD, Chicago, IL (University of Chicago)

8:15 am Developments and controversies in current knowledge and treatment of pulmonary hypertension
Sebastian Bonnet, PhD, MSc, Quebec, Canada (CRIUCPQ)

8:50 am Developments and controversies in current knowledge and treatment of ARDS
Stefan Uhlig, PhD, Aachen, Germany(University of Aachen)

9:25 am Biology of Endothelial Injury in Patients with Sepsis and ARDS
Michael Matthay, MD, San Francisco, CA (UCSF)

10:00 am Break (15 min)

10:15 am Regulation of Lung Endothelial Permeability and Inflammation by Actin Cytoskeleton
Steven Dudek, MD, Chicago, IL (UIC, Chicago)

10:50 am Endothelial Cell Junctions as Crossroads of Permeability and Inflammation
Asrar Malik, PhD, Chicago, IL (UIC)

11:25 am Dual role of calcium signaling in propagation and mitigation of Endothelial Dysfunction and ARDS
Mary Townsley PhD, Mobile, AL (University of South Alabama)

12:00 pm Lunch

Session II: Micro- and Macro-Environment Factors in Endothelial Dysfunction and ARDS Severity
Moderator: Eric Schmidt, MD

3:00 pm Terry Wagner Lecture:
Endothelial metabolic dysregulation, endothelial cell propensity for proliferation, and role of resident progenitor endothelial cells in ARDS
Mervin Yoder, MD, Indianapolis, IN (Indiana University)

3:40pm Cell-Cell Communication at Subcellular Level: Glycocalix and Microparticles
Eric Schmidt, MD, Denver, CO (University of Colorado School of Medicine)

4:15 pm Substrate Stiffness-dependent Exacerbation of Endothelial Permeability and Inflammation: Mechanisms and Potential Implication in ALI and PH
Konstantin Birukov, PhD, MD presenting on behalf of Anna Birukova, MD, Chicago, IL (University of Chicago)

COURSE SCHEDULE

4:50 pm Involvement of Cystic Fibrosis Transmembrane Conductance Regulator in Lung Endothelial Cell Barrier Dysfunction and Microvascular Damage from Cigarette Smoke
Irina Petrache, MD, Denver, CO (National Jewish Health)

5:20 pm Abstract Award Recipient:
Lung infection elicits endothelial amyloids with distinguishable antimicrobial and cytotoxic properties
Sarah Voth, BS, Mobile, AL (University of South Alabama)

5:40 pm Abstract Presentation:
Lung microvascular injury induced by cell-free hemoglobin is exacerbated by hyperoxia and inhibited by acetaminophen
Ciara M. Shaver, Nashville, TN (Vanderbilt University Medical Center)

6:00 pm Dinner

7:00 pm Meet the Professor Hour

Friday, September 8, 2017

Session III: Homeostatic and Drug-assisted Mechanisms of Lung Endothelial Repair and Resolution of ALI
Moderator: Steven Dudek, MD

7:00-8:00 am Breakfast

8:05 am Jack Reeves Lecture:
Lung repair mechanisms determined by cellular cross-talk with microvascular endothelium
Jahar Bhattacharya, MD, New York, NY (Columbia College of Physicians & Surgeons)

8:40 am Novel Regulators of Endothelial Barrier Function
Stefan Uhlig, MD, Aachen, Germany (University of Aachen)

9:15 am Injured Lung Endothelium: Mechanisms of Self-repair and Agonist-assisted Recovery
Konstantin Birukov, PhD, MD, Chicago, IL (University of Chicago)

9:50 am Abstract Award Recipient:
Role of Inflammation-induced Caveolin-1 and BMPRII Depletion in TGFβRI-mediated Pulmonary Vascular Remodeling
Suellen D'Arc dos Santos Oliveira, PhD, Chicago, IL (University of Illinois at Chicago)

10:05 am Break (10 min)

10:15 am Lung Vascular Targeted Drug Delivery: New Generation of Location- and Disease-State-Specific Targeting Vectors and Primary Demonstration of their Applications
Vladimir Muzykantov, MD, Philadelphia, PA (University of Pennsylvania)

10:50 am Late Morning Poster Session: Chaired by K. Weir, T. Stevens, S. Erzurum

12:00 pm Lunch

B. Endothelium in Pulmonary Hypertension and ILD: Can Remodeling be Stopped where it Starts?
Session IV: Role of Endothelium in Lung Vascular Remodeling Associated with Pulmonary Hypertension
Moderator: Vinicio de Jesus Perez, MD

3:30 pm Robyn Barst Lecture:
Endothelial-mesenchymal Transition in PH, Is It the Same in Newborn and Adults?
Kurt Stenmark, MD, Denver, CO (University of Colorado)

4:05 pm Endothelial Proliferation in PH
Rubin Tuder , MD, Denver, CO(University of Colorado)

4:40 pm Endothelial apoptosis as a key trigger in the development of PAH
R. James White, MD, PhD presenting on behalf of Duncan Stewart, MD, Rochester, NY (University of Rochester)

COURSE SCHEDULE

5:15 pm Abstract Award Recipient:
ROS-induced Ca2+ influx and microvascular endothelial dysfunction in the SU5416/Hypoxia model of pulmonary arterial hypertension (PAH)
Karthik Suresh, PhD, Baltimore, MD (Johns Hopkins University)

5:45 pm Abstract Presentation:
Redox Sensitive Sex Differences in 16αOHE2-Induced Migration and AhR Pathway Regulation in Human Blood Outgrowth Endothelial Cells
Katie Yates Harvey, PhD, Glasgow, UK (University of Glasgow)

6:00 pm Dinner

8:00 pm After Dinner Talk: Plexiform Lesions in PH – Where Do They Come From, and Are They Relevant?
Norbert Voelkel, MD, El Prado, NM (Emeritus

Saturday, September 9, 2017

Session V: Endothelial dysfunction in PH
Moderator: Sharon Rounds, MD

7:00-8:00 am Breakfast

8:05 am Endothelial Dysfunction in PH
Christophe Guignabert, MD, PhD, France (INSERM)

8:40 am The Role of Endothelial Leak in PH
Troy Stevens, PhD, Mobile, AL (University of South Alabama):

9:15 am Endothelial Dysfunction in PAH caused by Bone Morphogenetic Protein Receptor Type 2 Mutations
Mark de Caestecker, MBBS, PhD, Nashville, TN (Vanderbilt University)

9:50 am Break (15 min)

10:05 am Endothelial Inflammation in PH
Wolfgang Kuebler, MD, Ontario, Canada (University of Toronto)

10:40 am Group 3 PH: Stimulation of Soluble Guanylate Cyclase in pulmonary hypertension
Norbert Weissmann, PhD, Giessen, Germany (Universities of Giessen and Marburg Lung Center)

11:15 am Gender differences in endothelial-SMC interactions
Mandy MacLean, PhD, Glasgow, United Kingdom (University of Glasgow)

12:00 pm Lunch

Session VI: Mechanism of Impaired Endothelial Function in PH
Moderator: KModerator: Rubin Tuder, MD

3:00 pm Acute lung injury and the narrative of discovery: back to the beginning
David Cornfield, MD, Stanford, CA (Stanford University)

3:35 pm Reversing the endothelial dysfunction and vascular remodeling in pulmonary hypertension: dream or reality?
Vinicio de Jesus Perez, MD, Palo Alto, CA (Stanford University)

4:10 pm Estelle Grover Lecture:
Impaired Endothelial Metabolism and Its Role in PH
Serpil Erzurum, MD, Cleveland, OH (Cleveland Clinic)

4:45 pm Epigenetic Regulation of Endothelial Cells in Pulmonary Hypertension
Stephen Chan, MD, PhD, Pittsburgh, PA (Pittsburgh University)

COURSE SCHEDULE

5:20 pm Abstract Presentation:
Highly Efficient in-vivo Targeting of the Pulmonary Endothelium using Novel Modifications of Polyethylenimine
Andrew W. Dunn, MS, Cincinnati, OH (Cincinnati Children's Hospital Medical Center)

6:00 pm Dinner

7:00 pm Poster Session (evening): Chaired by N. Voelkel, J. Bhattacharya and M. Rabinovitch

Sunday, September 10, 2017

Session VII: Endothelium in COPD and Group 3 PH
Moderator: Rubin Tuder, MD

7:00-8:00 am Breakfast

8:05 am Chronic Obstructive Pulmonary Disease: A Disease of the Endothelium?
Caroline Owen, MD, PhD, Boston, MA
(Brigham and Women's Hospital)

8:40 am Pulmonary Microcirculation and Angiogenesis in Interstitial Lung Disease: Too Much or Not Enough?
Susan Majka, PhD, Nashville, TN
(Vanderbilt University)

9:15 am Abstract Award Recipient:
Nanoparticle-Mediated Delivery of STAT3 Stimulates Lung Angiogenesis
in Mouse Model of Alveolar Capillary Dysplasia
Arun Pradhan, PhD Cincinnati, OH (Cincinnati Children's Hospital Medical Center)

9:35 am Break (15 min)

9:50 am Priming of Pro-Inflammatory Signaling Mechanisms in Vascular Endothelium
by Environmental Factors: Role in Augmentation of ALI and COPD
Sharon Rounds, MD, Providence, RI (Brown University)

10:30 am Abstract Presentation:
Novel Imaging Approaches to Assess Focal Lung Function
and the Vascular Response to Acute Injury and Acute Hypoxia
Heather D. Jones, MD, Los Angeles, CA
(Cedars-Sinai Medical Center)

11:00 am Closing summary (10 min)

12:00 pm Lunch

The conference adjourns after lunch.

ABSTRACT PRESENTATIONS

Non Faculty Abstracts

Molecular Insights into the Cellular Mechanisms of Right Ventricle Cardiomyocyte Hypertrophic Remodeling in Pulmonary Hypertension

Mohammad Alhamaydeh, Neil Kelly, Alexander Hoyt, Stephanie Mutchler, Megan Yeung, Rishab Shetty, Jian Hu, Jeffrey Baust, and Imad Al Ghouleh

Heart, Lung, Blood, and Vascular Medicine Institute, Division of Cardiology, Department of Medicine, University of Pittsburgh School of Medicine

Introduction: There is a dearth of information on molecular pathways underlying the right ventricle (RV) hypertrophic remodeling (both adaptive and maladaptive) in pulmonary hypertension (PH) patients that occurs as a result of the associated sustained pressure overload. In this work, we uncovered a number of cellular mediators that converge on modulation of RV cardiomyocyte hypertrophic responses.

Methods: Cardiomyocyte-derived H9C2 cells and RV rat neonatal cardiomyocytes isolated from 1-day-old pup hearts (RV-RNCM) were subjected to neurohormonal hypertrophic stimulation using angiotensin II (AngII, 1 & 10 μM) and phenylephrine (PE, 1 & 10 μM). RV pressure overload was induced in mice by pulmonary artery banding (PAB; 3wk).

Results: AngII and PE treatment of H9C2 and RV-RNCM resulted in cellular hypertrophy, production of reactive oxygen species (ROS), and induction of NADPH oxidase 1 (Nox1) and NHE regulatory factor 1 (Nherf1) protein expression. The AngII-induced hypertrophy was attenuated by Nox1 and Nherf1 gene knockdown using siRNA. To test whether Nox1 and Nherf1 are linked, co-IP studies were performed between Nherf1 and the Nox organizer subunit p47phox revealing a Nherf1-p47phox association in H9C2 cells that is enhanced by prohypertrophic AngII-treatment. To investigate downstream mechanisms, loss of function experiments revealed that knockdown of apoptosis signal-regulating kinase 1 (Ask1) and the water channel aquaporin1 (Aqp1) by siRNA attenuated AngII-induced hypertrophy. In vivo, PAB induced an increase in RV ROS production and Nox1 and Nherf1 expression as well as Nherf1-p47phox association, supporting pre-clinical relevance of in vitro findings. This pathway appeared to have RV chamber specificity since aortic banding for 3 weeks did not affect left ventricle levels of ROS or Nox1.

Conclusion: The present study reveals a pro-hypertrophic cellular pathway in RV cardiomyocytes and introduces new potential therapeutic targets in PH by implicating a network of molecular mediators previously not connected to either the RV or PH.

A model of polymicrobial sepsis with high levels of circulating cell free hemoglobin causes lung endothelial apoptosis and permeability

Julie A Bastarache,[1] Ciara M Shaver,[1] Joel McNeil,[1] Nancy Wickersham,[1] James L Wynn,[2] and Lorraine B Ware[1]

[1]Department of Medicine, Vanderbilt University Medical Center, Nashville, TN and [2]Department of Neonatology, University of Florida.

Background: The majority of sepsis patients have elevated levels of circulating cell-free hemoglobin (CFH) but the consequences of this release of CFH are unknown. We developed a model of polymicrobial sepsis with high circulating CFH to test the hypothesis that CFH in sepsis injures the lung microvascular endothelium and contributes to pulmonary edema formation.

Methods: Mice were injected IP with a slurry of cecal contents from a donor mouse and IV with CFH (CS+CFH model). Clinical endpoints of severity of illness, weight loss and mortality were assessed for 4 days. Lung microvascular permeability was assessed by lung accumulation of a fluorescent macromolecule using high sensitivity imaging. Lung inflammation was assessed by BAL KC, total lung MPO and whole lung PCR for IL-6, TNF-a and KC. Endothelial injury markers (E-selectin, ICAM-1, PAI-1 and thrombomodulin,Tm) were measured in plasma. Lung endothelial apoptosis was assessed using co-immunostaining for TUNEL and Tm. In cultured HUVECs, we measured electrical resistance (ECIS) and macromolecular permeability (^{14}C-Inulin transfer) after CFH treatment.

Results: CS+CFH increases severity of illness, weight loss and mortality versus sham. Lung permeability, BAL KC, lung MPO, lung cytokine mRNA and lung endothelial apoptosis were all increased in the CS+CFH versus sham. In HUVEC's, CFH increased macromolecular permeability and decreased electrical resistance of monolayers.

Conclusions: In peritoneal sepsis with high levels of circulating CFH, lung permeability is increased, likely through induction of lung microvascular endothelial apoptosis. CFH alone can increase permeability in cultured endothelial cells and may represent a novel therapeutic target in sepsis. .

Endocytic Mechanisms and Intracellular Localization of Extracellular Vesicles (Microparticles) Interacting with Pulmonary Endothelium.

Michael Marfice, April K. Scruggs, and Natalie N. Bauer.

Department of Pharmacology and Center for Lung Biology, College of Medicine, University of South Alabama. Mobile, AL 36688.

Extracellular vesicles (EVs), which are largely composed of cellular membrane released microparticles (MPs), are membrane intact vesicles containing proteins, RNA, and second messengers. Circulating MPs contribute to pulmonary vascular injury in pulmonary hypertension and ARDS. However, the mechanisms of these detrimental interactions are not known. Previous work in our lab has shown that MPs co-localize with early endosomes and the trans golgi network following uptake by pulmonary microvascular endothelial cells (PMVECs), though the specific endocytic mechanism is undetermined. Caveolin- (CavME) and Clathrin-mediated endocytosis (CME) are two well-characterized mechanisms of endocytosis; therefore, both were examined as potential facilitators of MP entry. MPs were collected from the media of PMVECs, isolated by serial centrifugation, and labeled with PKH67, a fluorescent membrane marker. Naive PMVECs were pretreated with filipin (2μM; 1hr) or MDC (200μM; 1hr), inhibitors of CavME and CME, respectively. Inhibitor-treated or control PMVECs were then exposed to labeled MPs collected from $3x10^6$ cells for either 1 or 6 hours. Following MP treatment, PMVECs were fixed and labeled with Rab5 or TGN38, fluorescent antibodies for the early endosome and the trans golgi network. Treated PMVECs were analyzed in the z-plane by confocal microscopy to generate multiple images that were analyzed by CellProfiler to calculate the number of MPs and co-localization with organelles within PMVECs. MDC inhibited 90% of MP uptake and interaction with endosomes or the TGN, whereas filipin was not significant. Our results suggest the likely endocytic mechanism of MPs in PMVECs is CME-dependent and many MPs endocytosed by PMVECs follow the early endosomal pathway to the trans golgi network

Meprin-α: a glycosaminoglycan binding protease

Valentina Biasin[1], Malgorzata Wygrecka[2], Katharina Jandl[1], Zoltan Balint[3], Gabor Kovacs[1,4], Christoph Becker Pauly[5], Bahil Ghanim[1,6], Walter Klepetko[6], Andrea Olschewski[1, 7] Grazyna Kwapiszewska[1, 7]

[1] Ludwig Boltzmann Institute, Lung Vascular Research, Graz, Austria [2] Department of Biochemistry, University of Giessen, Germany [3] Faculty of Physics, Babeș-Bolyai-University, Cluj-Napoca, Romania, [4] Division of Pulmonology, Medical University of Graz, Austria [5] Institute of Biochemistry, University of Kiel, Germany [6] Division of Thoracic Surgery, Medical University of Vienna, Austria and [7] Institute of Physiology, Medical University of Graz, Austria

Pulmonary hypertension (PH) is characterized by increase pulmonary pressure and vascular remodelling as a consequence of smooth muscle cells proliferation and endothelial cells dysfunction. Meprin-α is a metalloprotease involved in cleavage of several substrate such as growth factors and ECM components, known to be involved in vascular remodelling. The aim of this study is to investigate the role of meprin-α in the remodelling process of PH. Meprin-α was elevated in plasma samples from PH patients in comparison to controls. Confocal microscopy revealed the presence of meprin-α in the luminal side of endothelial cell membrane as meprin-α spatially co-localized with the transmembrane N-terminal thrombomodulin. In vitro binding assay confirmed binding of meprin-α on human pulmonary artery endothelial cells (hPAEC) which was heparan-sulfate (HS) dependent. Binding of meprin-α to HS was confirmed by confocal microscopy and dot-blot. This finding was further confirmed in CHO cells and the mutant CHO pgsD-677 cell line (deficient in HS synthesis) where meprin-α binding was disrupted. Addition of meprin-α did not influence endothelial barrier integrity, as measured by electric impedance. In conclusion we reported for the first time binding of meprin-α to the HS of lung endothelial cells. We speculate that meprin-α can play a role in endothelial cell homeostasis; however the underlying mechanism needs further investigation.

MMP-8 Inhibits Vascular Remodeling and is Protective in Hypoxia-Induced Pulmonary Hypertension

Paul B Dieffenbach[1], Christina Mallarino Haeger[1], Anna Maria F. Coronata[1], Francesca Polverino[1], Sally H. Vitali[2], Robert F. Padera[1], Caroline A. Owen[1], Laura E. Fredenburgh[1]

[1]Brigham and Women's Hospital, Harvard Medical School, Boston, MA; [3]Boston Children's Hospital, Harvard Medical School, Boston, MA

Matrix metalloproteinase-8 (MMP-8) can regulate inflammatory and fibrotic responses to injury, and has been associated with pulmonary fibrosis and vascular atherosclerosis. Several MMPs have been implicated in PAH pathogenesis; however, the role of MMP-8 has not previously been investigated. Circulating MMP-8 levels were found to be 18-fold increased in PAH patients

compared to healthy controls (8.9±4.7ng/mL vs. 0.14±0.3ng/mL), and MMP-8 expression was increased in pulmonary arteries from PAH patients. MMP-8 expression was also elevated in PAs of MCT-treated rats and rats exposed to SU5416+hypoxia. Strikingly, MMP-8–/– mice showed increased mortality during exposure to hypoxia, with 50% survival at 8 weeks compared with 100% survival in WT mice (p=0.0008). MMP-8-/- mice developed significant elevation in right ventricular systolic pressure after 2 weeks of hypoxia and significant right ventricular hypertrophy (RVH) at 4 weeks of hypoxia compared with MMP8+/+ mice and normoxic controls. Echocardiography demonstrated RV dysfunction, and histology revealed increased PA wall thickness and severe RVH with RV dilation. PASMCs isolated from MMP-8-/- mice had significantly enhanced proliferation compared with cells derived from MMP-8+/+ mice, and ~ 3-fold increased activity of the pro-proliferative transcriptional activators YAP and TAZ. In summary, MMP-8 levels are increased in plasma and pulmonary arteries of PAH patients. Deficiency of MMP-8 in mice leads to increased mortality, RVH, and enhanced pulmonary vascular remodeling in response to hypoxia. This correlates with hyperproliferation of MMP8-/- PASMCs and enhanced YAP/TAZ transcriptional activity. MMP-8 may play a critical protective role in the pathobiology of PAH.

Highly Efficient *in-vivo* Targeting of the Pulmonary Endothelium using Novel Modifications of Polyethylenimine

Andrew W. Dunn[1], Donglu Shi[1],Vladimir V. Kalinichenko[2]

[1]The Materials Science and Engineering Program, Dept. of Mechanical and Materials Engineering, College of Engineering and Applied Sciences, University of Cincinnati, Cincinnati, Ohio 45221 and [2]Center for Lung Regenerative Medicine, Division of Pulmonary Biology and the Perinatal Institute, Cincinnati Children's Hospital Research Foundation, Cincinnati, Ohio 45229

Pulmonary vascular disease encompasses a wide range of serious afflictions with important clinical implications. There is a critical need for the development of targeted, efficient, non-viral gene therapy delivery systems for tailored treatment to reduce potentially dangerous off-target effects. We report a promising avenue to overcome the critical issues in cell targeting via uniquely designed nanosystems Nanoparticles are created by functionalizing low molecular weight polyethylenimine (PEI) or poly(ethylene glycol) (PEG) with biological fatty acids of varying chain lengths and degree of unsaturation through a one-pot EDC/NHS reaction. PEG is incorporated into PEI based colloids using microfluidic mixing or direct conjugation resulting in formulations possessing positive charge and small colloidal size upon binding to plasmids. This specific combination of material and polyplex properties has allowed for these novel formulations of cationic based, non-viral nanoparticles to efficiently target the pulmonary microvascular network for the delivery of nucleic acids. After injection into blood circulation, the nanoparticles show an exceptionally high specificity for the pulmonary microvascular endothelium with minimal targeting of other cell types in the lung. Live in-vivo imaging, flow cytometry of single cell suspensions, and confocal microscopy were used to demonstrate that polyplexes are enriched in the lung tissue, disseminated in 85 - 90 % of alveolar capillary endothelium while sparce in large vessels, and avoid association with other cell lineages.

A 17β-Estradiol (E2)-Estrogen Receptor α (ERα)-Apelin Axis Protects Against Right Ventricular (RV) Vascular Loss in Experimental Pulmonary Hypertension

Andrea Frump[1], Marjorie Albrecht[1], Sandra Breuils-Bonnet[2], Bakhtiyor Yakubov[1], Mary Beth Brown[1], Steeve Provencher[2], Sebastien Bonnet[2], Tim Lahm[1,3]

[1]Indiana University, [2]Université Laval, [3]Roudebush VA Medical Center

Introduction: Women with pulmonary arterial hypertension (PAH) exhibit better RV function and survival than men; however, the underlying mechanisms are unknown. Since decreased vascular density is a purported contributor to development of RV failure, we investigated the role of E2 on RV vascular density and cardiac endothelial cell (CEC) function.

Hypothesis: E2, through ERα, attenuates PH-induced RV capillary loss by up-regulating the pro-angiogenic peptide apelin.

Methods: ERα and apelin were analyzed (western blot) in RV homogenates from PAH patients, male or female Sprague-Dawley rats with sugen/hypoxia (SuHx)-PH (subsets were ovariectomized+/-E2 repletion) or hypoxic PH (HPH), ERα or ERβ null mice with HPH and in human CECs treated with E2 or ERα agonist (0.1-100nM; 2-24 hrs). Capillarization of SuHx-RVs was determined by lectin staining. E2 (0.1-100nM; 6hrs) effects on CEC tube formation were assessed by matrigel assay. $p<0.05$ was significant.

Results: RV apelin abundance was decreased in RVs with maladaptive (SuHx) but not adaptive remodeling (HPH; $p<0.05$). E2 protected against capillary loss in SuHx-RVs and increased tube formation in CECs, findings accompanied by increased apelin expression in SuHx-RVs and CECs ($p<0.05$). Treatment with ERα agonist recapitulated E2's effects on apelin abundance in vivo (SuHx-RV) and in vitro (CECs). In ERα or ERβ null mice with HPH, ERα was necessary for E2 to increase RV apelin ($p<0.05$). In SuHx or human PAH RVs, apelin expression correlated positively with ERα.

Conclusions: We identified a novel cardioprotective E2-ERα-apelin axis, which promotes angiogenesis and protects against vascular dropout in the RV. Harnessing this axis may lead to novel, RV-targeted therapies.

Early Pulmonary Vascular Disease in Young Adults Born Premature

Arij Beshish[1,3], Kara Goss[1,2], Kristin Haraldsdottir[1,3], Greg Barton[1,3], David Pegelow[1,3], Mari Palta4, Luke Lamers[1], Marlowe Eldridge[1,3]

Department of Pediatrics[1], Department of Medicine[2], John Rankin Laboratory of Pulmonary Medicine, University of Wisconsin – Madison[3] and Department of Biostatistics and Medical Information, University of Wisconsin – Madison[4]

Introduction: Preterm birth has recently been associated with a 4-fold increase in the risk for development of adult pulmonary hypertension, yet the mechanisms behind this association are poorly understood. Here, we sought to characterize the pulmonary vascular response to exercise in young adults born premature.

Methods: Healthy adults born preterm were recruited from the Newborn Lung Project, a prospectively enrolled cohort of very low birth weight infants (<1500 g) born from 1988-1991 (n=10), and age matched controls (n=9) were recruited from the general population. Subjects underwent placement of a Millar conductance catheter in the right ventricle and pulmonary artery, and pressures were recorded at rest and during submaximal exercise. Subjects also completed a cardiac MRI. Results were analyzed by t-test for rest and exercise conditions, and by 2-way ANOVA for overall effects of birth.

Results: Adults born premature had significantly higher mean pulmonary artery pressures at rest (18.4±4.1 vs 14.7±2.0 mmHg, p=0.03) and during exercise (21.4±4.5 vs 17.4±2.7 mmHg, p=0.03). Cardiac output, stroke volume, and heart rate responses to exercise were similar between groups. Although total pulmonary resistance was similar at rest, during exercise adults born preterm had significantly higher total pulmonary resistance, lower capacitance, and lower pulmonary artery relative area change (p<0.05 for all).

Conclusions: Healthy adults born premature demonstrate evidence of early pulmonary vascular disease, including mild elevations in pulmonary artery pressure at rest and during exercise. The elevated total pulmonary resistance, lower capacitance and lower relative area change suggest a stiffer less recruitable vascular bed.

Redox Sensitive Sex Differences in 16αOHE2-Induced Migration and AhR Pathway Regulation in Human Blood Outgrowth Endothelial Cells.

Katie Yates Harvey[1], Nicholas W Morrell[2], Margaret R MacLean[1].

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Sex and sex hormones appear to play an important role in pulmonary arterial hypertension (PAH). Preliminary unpublished data from our lab suggests a role for 16alpha–hydroxyestradiol (16αOHE2) in male PAH. Recent evidence suggests dysfunctional aryl hydrocarbon receptor (AhR) signalling could influence sex hormone synthesis and metabolism and may be involved in endothelial dysfunction in PAH.

Here we investigate the effects of 16αOHE2 in male and female control and PAH patient derived blood outgrowth endothelial cells (BOECs) (n=3). Migration was assessed using transwell inserts and RT-PCR used to analyse expression of AhR pathway genes. As AhR is activated by translocation from the cytoplasm to the nucleus, we assessed AhR nuclear translocation by the REAP method of cell fractionation and immunoblotting. To investigate redox signaling, we studied the effects of Bardoxolone methyl, a nuclear factor erythroid-2-related factor-2 (Nrf-2) activator.

16αOHE2 (10-9M) increased migration (183±4%; p<0.05), in male but not female PAH BOECs. Bardoxolone (10-8M), attenuated 16αOHE2-induced migration in male PAH BOECs (134±5%; p<0.05). Basally, cytochrome P450 1A1 (CYP1A1) mRNA transcript was reduced in male PAH versus control BOECs. In male control BOECs, 16αOHE2 decreased mRNA levels of Aryl hydrocarbon receptor nuclear translocator (ARNT) and CYP1A1 and AhR gene expression and nuclear translocation, whereas in male PAH BOECs, 16αOHE2 increased AhR transcript expression and nuclear translocation.

In conclusion, male, but not female PAH BOECs are sensitive to 16αOHE2-induced migration, involving redox sensitive mechanisms. Dysregulated AhR signalling and activation by 16αOHE2 may contribute to endothelial dysfunction in PAH in men.

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Mitochondrial uncoupling protein 2 regulates the lung endothelial barrier

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Reactive oxygen species (ROS) induce expression of mitochondrial uncoupling protein 2 (UCP2) (PMID 26198983), which blunts further ROS production by negative feedback (PMID 25910810). Following airway acid instillation, a procedure that models acid aspiration-induced acute lung injury (AALI), contact between acid and alveolar epithelium (AE) initiates AE-endothelial ROS transfer (PMID 22561462). We considered endothelial responses that might underlie AALI. We viewed isolated blood-perfused mouse lungs by confocal microscopy. To determine endothelial mitochondrial polarization and barrier properties, we infused lung microvessels with the potentiometric mitochondrial dye, TMRE, and fluorescently labeled 70 kD dextran (FD70). To determine acid effects, we microinstilled HCl (pH 1.1) by micropuncture of the alveolar lumen. We confirmed that microinstilled acid did not leak from alveoli. Baseline images indicated that TMRE fluorescence was stable. However, 10 min after acid instillation, endothelial TMRE fluorescence decreased markedly (n=4, $P<0.05$), indicating the onset of mitochondrial depolarization. Pretreating microvessels with N-acetyl cysteine, or with siRNA to specifically knockdown endothelial UCP2 (UCP2 KD), blocked the endothelial TMRE loss (n=3, $P<0.05$). At baseline, FD70 fluorescence was restricted to the microvascular lumen, indicating the endothelial barrier was viable. However, acid microinstillation induced FD70 leakage from microvessels to the alveolar lumen (n=4, $P<0.05$), indicating the barrier was hyperpermeable. UCP2 KD blocked the hyperpermeability response. We interpret, alveolar acid injection led to paracrine transfer of epithelial ROS to adjoining endothelia, activating UCP2, hence depolarizing endothelial mitochondria. Mitochondrial depolarization caused endothelial barrier deterioration. Our findings are novel evidence that AALI results from rapid UCP2-dependent loss of endothelial barrier properties. (Support: NIH T32 HL105323)

Novel Imaging Approaches to Assess Focal Lung Function and the Vascular Response to Acute Injury and Acute Hypoxia

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Direct imaging of the murine pulmonary vasculature requires invasive, terminal procedures and cannot interrogate the global and regional responses to injury. We used phase-contrast X-ray imaging and novel image analysis techniques (4DxV and contrast-free pulmonary angiography, CFPA) to assess the vascular responses to acute lung injury and acute hypoxia. 4DxV analysis provided 3D high-resolution imaging of regional lung ventilation. CFPA analysis of non-contrast scans generated 3D vascular trees, and diameters of vessels at >10,000 locations/mouse were determined. In response to acute injury, tissue ventilation was markedly decreased compared to baseline, and vascular diameters increased significantly. In response to acute hypoxia, the proportion 100-150-μM-diameter vessels decreased (possibly reflecting a shift towards vessel diameters below the limit of resolution) and returned to baseline within 30 min of normoxia. These findings demonstrate that novel X-ray-based imaging and analysis provides high-resolution images of the lungs in mice and yields detailed quantitative data on vascular responses. This technology holds great promise for multiple applications in preclinical models of lung disease.

Caveolin-1 Loss in Neointimal Cells: Cause of Irreversible Pulmonary Hypertension?

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Despite modern therapy, pulmonary hypertensive changes progress unabated. We have shown that unlike the hypoxia model, monocrotaline (MCT) injection in rats induces progressive endothelial caveolin-1 (cav-1) loss and reciprocal activation of proliferative pathways leading to pulmonary hypertension (PH). At 2 weeks post-MCT, low cav-1 expression coupled with relatively normal eNOS expression results in superoxide generation which returns to normal by 3 weeks as eNOS expression decreases. In order to examine the mechanism of PH irreversibility, we exposed MCT- treated rats to hypoxia (M+H) starting on day 1. This resulted in the acceleration of the disease process. At 4 weeks, there was significantly increased pulmonary artery pressure and right ventricular hypertrophy compared with MCT and hypoxia alone groups. In the M+H group, extensive endothelial damage and endothelial cav-1 loss, enhanced cav-1 expression in SMC, and neointimal lesions were observed. Neointimal cells stained positive for smooth muscle α-actin, and exhibited scant cav-1 expression. However, eNOS expression was normal. The expression of Nrf2, a factor that regulates the adaptive stress response and cell survival was significantly increased in M+H group. In addition, MnSOD and glucose transporter (Glut) 1 expression was increased. During oxidative stress, Nrf2 is activated; it translocates to the nucleus, inducing several antioxidant factors including MnSOD. It inhibits oxidative stress-induced activation of p53 and facilitates aerobic glycolysis. Cav-1 is known to directly

bind to Nrf2, and inhibit its translocation to nucleus, and facilitate p53 activation, thereby inhibiting cell proliferation. In addition, cav-1 modulates Glut1 expression. In conclusion, neointimal cav-1 loss leads to the activation of Nrf2 and its downstream factors, and increased Glut1 expression which provide pro-survival milieu for the proliferating cells leading to irreversible PH.

eNOS Uncoupling in Absence of Caveolin-1 Promotes Pathological Angiogenesis

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Reduced expression of caveolin-1 (Cav1) in endothelial cells (ECs) is associated with endothelial-to-mesenchymal transition (EndoMT) and pulmonary vascular remodeling. Pulmonary vascular pathology in Cav-1-/- mice is prevented when eNOS is also deleted (i.e., double knockout mice). We hypothesized that eNOS-derived oxidants in absence of Cav-1 results in aberrant postnatal angiogenesis. Mouse lung ECs (MLECs) isolated from WT, Cav1-/-, and Cav1-/-;eNOS-/- mice were used to assess gene and protein expression, NO vs. peroxynitrite levels, and to quantify indices of sprouting angiogenesis in vitro and upon transplantation in vivo. Video microscopy of Cav1-/-;Flk1-/+GFP knock-in primary MLECs showed increased sprouting and tip-bifurcations, reduced junctional VE-cadherin and CD31 staining, and reduced potential to form tubules with lumens compared to WT;Flk1-/+GFP MLECs. Moreover, the defect in tube formation was abolished in Cav1-/-;eNOS-/-. In support of these findings, we observed elevated levels of peroxynitrite and reduced Notch-1 cleavage, NICD accumulation in the nucleus, and expression of Notch effector Hey-1 in Cav-1-/- but not Cav1-/-;eNOS-/- MLECs. We also observed reduced Flk1 and Notch-4 mRNA levels and increased Notch-2 and -3 gene expression in mesenchymal-like Cav-1-/- but not Cav1-/-;eNOS-/- MLECs. Finally, treatment of Cav1-/-;eNOS-/- MLECs with peroxynitrite donor SIN-1, like the gamma-secretase inhibitor DAPT, reduced Notch-1 cleavage and induced EndoMT. Thus, eNOS-derived peroxynitrite in absence of Cav-1 promotes pathological angiogenesis by inhibiting Notch-1 and Notch-4 signaling and leading to disorganized and unproductive EC outgrowths.

Role of Inflammation-induced Caveolin-1 and BMPRII Depletion in TGFβRI-mediated Pulmonary Vascular Remodeling

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Caveolin-1 (Cav-1) is highly expressed in pulmonary microvascular ECs and plays a key role in maintaining vascular homeostasis. The aim of this study was to determine whether acute and chronic lung inflammation promotes Cav-1 depletion and whether this sensitizes the pulmonary vasculature to profibrotic signals such as TGF-β. C57BL6 WT mice (Tie2.Cre-;Cav1$^{lox/lox}$) exposed to nebulized LPS (10 mg; 1 hr daily for 1 and 4 days) or hypoxia (for 1 month) were compared to EC-specific Cav1$^{-/-}$ (Tie2.Cre+;Cav1$^{lox/lox}$). After 4 days of LPS exposure, total lung Cav-1 and BMPRII expression were reduced in WT mice. Moreover, plasma albumin leakage, infiltration of immune cells, and levels of IL-6/IL-6R and TGF-β were elevated in both LPS-treated WT and EC-Cav1$^{-/-}$ mice. Finally, EC-Cav1$^{-/-}$ mice exhibited a modest increase in microvascular thickness basally and moreso upon exposure to LPS or hypoxia. EC-Cav1$^{-/-}$ mice and LPS treated WT mice exhibited eNOS uncoupling and increase in reactive nitrogen species production, reduced BMPRII expression, which along with increased TGF-β, promoted TGFβRI-dependent SMAD-2/3 phosphorylation. Depletion of EC Cav-1 was associated with an increase in circulating Cav-1+ extracellular vesicles (EVs) 24 hrs after LPS exposure. Finally, human lung sections from patients with severe lung injury displayed reduced EC Cav-1 expression, elevated TGF-β levels, and severe pulmonary vascular remodeling. Thus, EC Cav-1 depletion, oxidative stress-mediated reduction in BMPRII expression, and enhanced TGF-β-driven SMAD-2/3 signaling may promote pulmonary vascular remodeling in inflamed lungs.

Post-septic pulmonary endothelial eparin sulfates acquire increased 6-O sulfation via downregulation of endothelial sulfatase-1

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Rationale: The endothelial glycocalyx is a heparan sulfate-rich layer that projects from the cell membrane into the vascular lumen. Pulmonary endothelial glycocalyx integrity is critical for endothelial homeostasis, and its septic degradation leads inflammatory lung injury. Given this critical importance, post-septic recovery of the glycocalyx is likely to contribute to reestablishing endothelial function.

We have recently reported that the glycocalyx reconstitutes itself in 72 hours in animal model of sepsis. However, the composition of the reconstituted glycocalyx, and whether it is consistent with what existed prior to degradation are unknown.

Methods: We induced sepsis in male C57BL/6J mice with cecal ligation and puncture (CLP). We determined glycocalyx composition by performing HPLC-mass spectrometry on the glycocalyx harvested from isolated perfused lungs. To explore enzymes responsible for changes in heparan sulfate sulfation, we flow-sorted pulmonary endothelial cells and performed RNA microarray. The mRNA expression of the target protein in pulmonary endothelial cells was further quantified by qRT-PCR.

Results: Mass spectrometric analysis showed that the post-septic pulmonary endothelial heparan sulfates had increased 6-O sulfation of glucosamine residues at 72 hrs ($P < 0.05$). Microarray analysis of flow-sorted endothelial cells (harvested 48 hrs after CLP) detected downregulation of sulfatase-1, a key enzyme that specifically cleaves 6-O sulfate ($P < 0.05$). In contrast, 6-O sulfation of heparan sulfates at 120 hrs, as well as the mRNA expression of sulfatase-1 (at 96 hrs) were not different between sham and CLP mice.

Conclusion: Our data suggest that endothelial heparan sulfates acquire a transient increase in 6-O sulfation by downregulation of sulfatase-1. The biological roles of sulfatase-1 in pulmonary endothelial cells will be investigated in future studies.

Nanoparticle-Mediated Delivery of STAT3 Stimulates Lung Angiogenesis in Mouse Model of Alveolar Capillary Dysplasia

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Alveolar Capillary Dysplasia with Misalignment of Pulmonary Veins (ACD/MPV) is a severe congenital disorder of neonates and infants, which is uniformly fatal and lacks effective treatment options. ACD/MPV is characterized by the loss of alveolar capillaries and is linked to mutations in FOXF1 gene. In the present study, we used CRISPR/Cas9 technology to generate the first clinically relevant mouse model of ACD/MPV by introducing the S52F FOXF1 point mutation, which was found in ACD/MPV patients, into the mouse Foxf1 gene locus. We demonstrated that this mutation disrupts STAT3-FOXF1 protein-protein interactions that are required for transcriptional regulation of many endothelial-specific genes. The S52F FOXF1 mutant exhibited diminished DNA binding and reduced transcriptional activity as measured by ChIP and dual luciferase assays. In cultured endothelial cells and in vivo, FOXF1 induced STAT3 signaling and activated transcription of STAT3 gene, a critical transcriptional regulator of endothelial proliferation. The S52F-Foxf1 knock-in mice exhibited a loss of lung microvasculature, decreased endothelial cell proliferation, diminished STAT3 signaling and increased mortality after birth, recapitulating histopathological findings in ACD/MPV patients. Finally, nanoparticle-mediated gene delivery of STAT3, which targeted microvascular endothelial cells, restored endothelial proliferation and stimulated lung angiogenesis in S52F-Foxf1 mutant mice. Altogether, the S52F-Foxf1 knock-in mice represent an excellent mouse model of ACD/MPV, which was used to demonstrate that STAT3 gene therapy is a promising strategy to treat ACD/ MPV patients.

Lung microvascular injury induced by cell-free hemoglobin is exacerbated by hyperoxia and inhibited by acetaminophen

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Introduction: Elevated pre-operative plasma cell-free hemoglobin (CFH) is an independent risk factor for primary graft dysfunction (PGD) after lung transplantation, an association that is augmented by high reperfusion FIO2. We hypothesized that oxidation of CFH in the presence of hyperoxia contributes to PGD by increasing lung microvascular permeability.

Methods: Isolated perfused human lungs were exposed to circulating CFH in the presence of normoxia (FIO2 0.21) or hyperoxia (FIO2 0.95). Microvascular permeability was assessed by change in lung weight and transit of Evans blue-labeled albumin dye (EBD) from perfusate into bronchoalveolar lavage. To test the direct endothelial effects of CFH, pulmonary microvascular endothelial cells (hPMVECs) were exposed to CFH (normoxia vs. hyperoxia) and paracellular permeability assessed by electrical cell-substrate impedance sensing. To determine whether acetaminophen (APAP), a specific hemoprotein reductant, could abrogate CFH-mediated microvascular injury, human lungs or hPMVECs were exposed to CFH or CFH+APAP.

Results: Exposure of human lungs to intravascular CFH with normoxia or to control with hyperoxia did not increase vascular permeability. In contrast, CFH combined with hyperoxia markedly increased weight gain and leak of EBD into the airspace (CFH+hyperoxia: 4.5% weight gain, EBD 12.3mcg/mL, CFH+normoxia: 1.2% weight loss, EBD 5.6mcg/mL; $p=0.043$ for each). In cultured hPMVECs, CFH increased paracellular permeability, an effect augmented 2.3-fold by hyperoxia ($p<0.05$). Clinically relevant

doses of APAP prevented CFH+hyperoxia-dependent increases in lung weight and EBD in human lungs ($p<0.046$) and reduced paracellular permeability of CFH-treated hPMVECs ($p=0.037$).

Conclusion: Oxidation of CFH in the presence of hyperoxia increases lung microvascular permeability through direct effects on endothelial cells that are inhibited by the hemoprotein reductant activity of acetaminophen.

ROS-induced Ca2+ influx and microvascular endothelial dysfunction in the SU5416/Hypoxia model of pulmonary arterial hypertension (PAH)

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In PAH, endothelial cells (ECs) exhibit increased migration and proliferation leading to formation of vaso-occlusive lesions. The mechanisms underlying EC phenotype switching remain unclear. Mitochondria are dysfunctional in human PAH ECs and oxidant stress and intracellular Ca2+ concentration ([Ca2+]i) are increased, yet the links between mitochondrial function, ROS production, elevated [Ca2+]i and enhanced migration and proliferation in PAH ECs are incompletely understood. To elucidate the possible role of ROS-induced Ca2+ influx in promoting migration and proliferation in PAH ECs, we isolated microvascular ECs (MVECs) from the lungs of rats subjected to normoxia (N-MVEC) or SU5416/hypoxia (SuHx-MVEC), a robust model of PAH. SuHx-MVEC appeared morphologically abnormal, with larger size, spindle-shaped rather than “cobblestone” morphology. While both N- and SuHx-MVEC stained positive for MVEC markers, SuHx-MVEC also exhibited smooth muscle markers. Examining mitochondrial morphology using Mitotracker and MitoRFP revealed that fission was increased in SuHx-MVEC, with increased mitochondrial number and fragmentation, decreased oxidative phosphorylation (measured via the SeaHorse Flux Analyzer) and activation of the fission-inducing protein dynamin-related protein 1. ROS levels, measured using roGFP, were increased in SuHx-MVEC, but attenuated following treatment with MitoQ (MQ) a mitochondrion-specific antioxidant. Basal [Ca2+]i (measured using Fura-2AM), migration (via transwell assay) and proliferation (via BrdU incorporation) were increased in SuHx-MVEC, but attenuated following treatment with MQ, a global ROS scavenger (TEMPOL) or HC-067047, a specific TRPV4 inhibitor. These data suggest SuHx-MVEC may undergo endothelial-mesenchymal transition in the context of mitochondrial fission and ROS production. Our data also suggest mitochondrial ROS activate TRPV4 in SuHx-MVEC, increasing basal [Ca2+]i and enhancing migration and proliferation.

P53 and HIF Signaling Crosstalk in Endothelial Cells Contributes to the Development of Pulmonary Hypertension

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Rationale: Pulmonary arterial hypertension (PAH) and carcinogenesis share numerous pathogenic features including altered crosstalk between cells, abnormalities of various growth factors and pathways, metabolic shifts, and inflammatory disorders. The tumor-suppressive role of p53, a transcription factor that regulates the expression of stress response genes, has been extensively investigated in malignant conditions. Our hypothesis is that a divergent change of p53 in pulmonary arterial smooth muscle cells (PASMC) and pulmonary arterial endothelial cells (PAEC) may crosstalk with hypoxia inducible factors (HIFs) signaling and link to the development of pulmonary hypertension (PH).

Methods: Ubiquitous MDM2 transgenic mice (MDM2TG), MDM2 Mutant transgenic mice (MDM2mut-TG), and wildtype (WT) littermates (6-8 weeks) were exposed to persistent chronic hypoxia (10% O2) for 21 days to establish hypoxia-induced PH. The pulmonary arterial pressure was measured using the isolated perfused/ventilated mouse lung system to determine hypoxic pulmonary vasoconstriction in mice.

Results: Compared to controls, decreased p53 expression was found in PA after 3 weeks of hypoxic exposure, while whole lungs displayed increased p53 expression in hypoxia-induced PH mice. In MDM2mut-TG mice, the hypoxia-mediated increase in RVSP and Fulton index (RV/(LV+S)) were significantly attenuated compared to WT mice. However, MDM2TG mice spontaneously develop mild PH under normoxic conditions and after exposure to chronic hypoxia develop more profound elevations of both RVSP and RVH compared to both WT and MDM2mut-TG mice.

Conclusions: These results indicate that p53 in endothelial and smooth muscle cells display differential responses to hypoxia and may play different roles in the development of PH. MDM2-p53 signaling may crosstalk with HIF signaling and be involved in the transcriptional regulation under hypoxia and play critical roles in the development of PH. Therefore, further studies are needed to identify the potential contribution of MDM2-p53 signaling in different types of pulmonary vascular cells.

Lung infection elicits endothelial amyloids with distinguishable antimicrobial and cytotoxic properties

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Nosocomial pneumonia induces lung endothelial amyloid production. These amyloid species are cytotoxic, self-replicating, and transmissible. They are insensitive to boiling, proteases, RNAse, and DNAse. Thus, infection-induced cytotoxic endothelial amyloids represent a form of prion disease, and emerging evidence suggests endothelial amyloids contribute to end-organ dysfunction in the aftermath of critical illness. However, mechanisms of host-pathogen interactions underlying production of endothelial amyloids remain unclear. Here, we tested the hypothesis that Pseudomonas aeruginosa type III secretion system (T3SS) effectors are sufficient for production of cytotoxic amyloids. To test this hypothesis, P. aeruginosa strains with (PA103 and PA01) and without (ΔPcrV) a functional T3SS were used to infect pulmonary microvascular endothelial cells (PMVECs) for 4 hours at an MOI of 20:1. Supernatants were collected, centrifuged, filter sterilized, and transferred to naïve PMVECs. Supernatant obtained from both PA103- and PA01-infected cells was cytotoxic. Amyloid antibody neutralization abolished cytotoxicity, and cytotoxicity was restored by adding back eluted amyloids. In contrast, supernatant obtained from ΔPcrV-infected cells was not cytotoxic. Consequently, we examined the antimicrobicity of infection-induced endothelial amyloids. Standard Kirby-Bauer antibiotic sensitivity assays were used with disk inoculants standardized to 10 µg/20 µL. Amyloids were visualized by Congo red staining. In contrast to the cytotoxicity assay, ΔPcrV-derived supernatant effected extensive bacteriostasis, whereas PA103- and PA01-derived supernatant had little effect. Bacteriostasis progressively increased over a 72-hour time course, and was suppressed by amyloid neutralization. These data indicate that T3SS effectors promote endothelial amyloid cytotoxicity while, provocatively, suppressing/abolishing antimicrobicity. This work provides opportunity to enrich novel antimicrobial compounds as therapeutics for antibiotic resistant organisms.

High altitude hypoxia impacts omega-3 fatty acid metabolites in plasma of fetal and newborn sheep

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Perinatal hypoxia has profound effect on the infant's pulmonary vascular development, with physiological impairment of pulmonary arterial function and structure. The present study explored the effect of chronic hypoxia during gestation and after birth on lipid mediator by measuring the metabolites that foreshadow oxidative stress and inflammation, which are primary drivers of pulmonary vascular dysfunction. We tested the hypothesis that chronic hypoxia reduces the amount of oxylipin and endocannabinoid production, which are important mediators of oxidative stress and inflammation. To test this hypothesis we exposed pregnant sheep and newborn lambs to an altitude of 3,800 meters starting gestation day 30. UPLC-MS/MS analysis was used to investigate the lipid mediator composition in plasma collected from veins of fetal and newborn animals. The results show that hypoxia causes an overwhelming effect on omega-3 fatty acids and their derivatives, which are crucial in late-stage fetal development. We tracked the origin of these changes and traced the pathways of several oxylipins and endocannabinoids. The cytochrome P450 (CYP) pathway enzymes and the subsequent activity of soluble epoxide hydrolase (sEH) are prominent synthesizers of epoxyeicosatrienoic acids (EETs) and epoxyoctadecenoic acids (EpOMEs), which can then be hydrolyzed into oxylipins. Previous studies have suggested the role of EETs and EpOMEs in vasodilation, a feature in inflammation. Based on these findings we demonstrated that a majority of the affected oxylipins were produced from CYP and sEH enzymes, which are important to pulmonary vascular function. These findings provide a novel insight into our understanding of lipid metabolites in hypoxia-induced pulmonary vascular dysfunction in the developing fetus and newborn and may help us develop novel therapies that target inflammatory pathways induced by pre and post-natal hypoxia. (Supported by NIH grants HD083132 [LZ], 1U24DK097154 [OF])

ROS-induced Ca2+ influx and microvascular endothelial dysfunction in the SU5416/Hypoxia model of pulmonary arterial hypertension (PAH)

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Hypoxia is a condition when body tissues are deprived of enough oxygen supply. Hypoxia alone can cause pulmonary vasoconstriction and significantly structural remodeling of pulmonary arteries, leading to pulmonary hypertension. At the molecular level, previous studies have linked hypoxia with dysregulation of small non-coding RNAs such as miRNA. However, with recent expanding discoveries of novel small RNA categories, such as ribosomal RNA-derived small RNAs (rsRNAs) and transfer RNA-derived small RNAs (tsRNAs) that that bear versatile functions, it's tempting to test whether hypoxia can also change the profile of these novel small RNAs. To this end, we developed a computational framework, SPORTS1.0 (small RNA annotation pipeline optimized for rRNA- and tRNA- derived small RNAs), which can comprehensively annotates various types of small RNAs, with particular optimization for discovery and profiling of tsRNAs and rsRNAs from sncRNA-seq data. Using SPORTS1.0, here we discovered that in addition to previously know miRNA changes, hypoxia induced significant changes in both rsRNAs and tsRNAs in mesenchymal stem cells (MSCs) cultured under hypoxic versus normoxia condition. These pilot results have demonstrated the feasibility of SPORTS1.0 in discovering novel small RNA signatures for various pathological conditions; and in present case, paving avenues for future studying the function of tsRNAs and rsRNAs in hypoxia and related pulmonary diseases.

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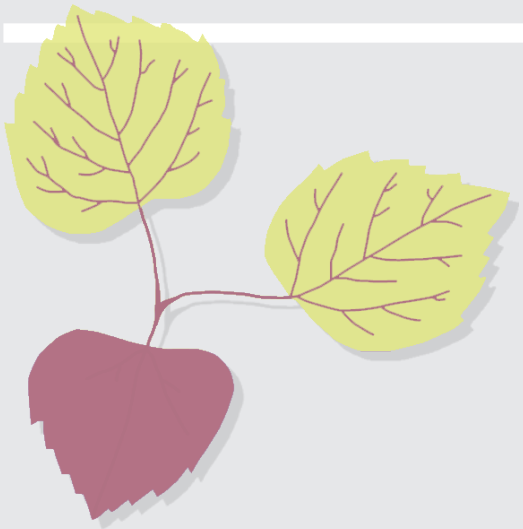

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