American Thoracic Society

ASSEMBLIES

GROVER CONFERENCE

2015

September 9-13, 2015

Lost Valley Conference Center, Sedalia, CO

The American Thoracic Society and the conference organizing committee gratefully acknowledge the educational grants provided for the support of this conference by Actelion Pharmaceuticals US, Inc., Gilead Sciences, Inc., Lung Biotechnology, and United Therapeutics Corporation.

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Additionally, the American Thoracic Society is grateful for the support of the Grover Conference by the American Heart Association, the Cardiovascular Medical Research and Education Fund, and the National Institute of Health.

THE PROGRAM

Since its inauguration in 1984, the 2015 Grover Conference will be the 17th 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 the interaction between the right ventricle and the pulmonary circulation as it occurs during development, in normal physiology and in disease states, notably pulmonary hypertension and congenital heart disease. The aim of the Grover Conference is to integrate 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 the manner in which recent novel findings, such as rare and common variants in certain genes, relate to the broader investigation of pulmonary circulation-related abnormalities such as pulmonary hypertension.
2. Incorporate '-omic' analyses directly into their scientific approach, in a manner that builds upon previous published and/or presented data, to provide a deeper level of phenotypic assessment of research subjects and/or cell or animal model systems.
3. Measure and integrate various '-omics' metrics together, to provide a more complete understanding of the normal and pathophysiologic processes which contribute to disease pathogenesis.
4. Design and implement an '-omics' –based proposal using one or more of the emerging technologies, such as next generation DNA and RNA sequencing, metabolomics assessments, etc…

Who Should Attend

Physician-scientists and scientists focused on the study of the pulmonary circulation and right ventricle, using basic science and translational approaches. Those with particular expertise in, or incorporating, '-omics' technologies will be highly encouraged to attend and engage in discussion. We anticipate wide interest across the spectrum of pulmonary circulation scientists.

PROGRAM COMMITTEE

Eric Austin, MD, MSCI, Chair, Organizer
Wendy K. Chung, MD, PhD
C. Gregory Elliott, MD
D. Hunter Best, PhD

SPEAKERS AND SESSION CHAIRS

Steven H. Abman, MD, University of Colorado, Denver, Denver, CO

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Christopher Baker, MD, University of Colorado, Denver, CO

Hunter Best, PhD, University of Utah School of Medicine, Salt Lake City, UT

Evan Brittain, MD, MSc, Vanderbilt Universtiy, Nashville, TN

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

Raed Dweik, MD, Cleveland Clinic, Cleveland, OH

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Hakon Hakonarson, MD, PhD, Children's Hospital of Philadelphia, Philadelphia, PH

Rizwan Hamid MD, PhD, Vanderbilt Universtiy, Nashville, TN

Jane Leopold, MD, Brigham and Women's Hospital, Boston, MA

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Marlene Rabinovitch, MD, Max-Plank Institute for Heart and Lung Research, Vanderbilt University, Nashville, TN

Larissa Shimoda, PhD, John Hopkin's University, Baltimore, MD

Florent Soubrier, MD, PhD, INSERM, Paris, France

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

Norbert Voelkel, MD, Virginia Commonwealth University, Richmond, VA

E. Kenneth Weir, MD, University of Minnesota, Minneapolis, MN

James West, PhD, Vanderbilt University, Nashville, TN

Jason X.-J. Yuan, MD, PhD, University of Illinois Chicago, Chicago, IL

2015 GROVER CONFERENCE on Pulmonary Circulation in the "omics" era: New Insights into Pathogenesis

COURSE SCHEDULE

Wednesday, September 9, 2015

12:00 pm **Arrivals**

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

7:00 pm **State of the Art: Translational Research in Pulmonary Vascular Disease: Challenge and Opportunity**
Norbert Voelkel, MD Taos, New Mexico and Denver, Colorado

Thursday, September 10, 2015

Session I: Systems Biology, Bioinformatics Integration, and the Process of Pulling it all Together for Cardiopulmonary Diseases
Moderator: Jason X.-J. Yuan, M.D., Ph.D.

7:00-8:00 am Breakfast

8:05 am Welcome and Introduction
Eric Austin, MD MSCI, Nashville, TN (Vanderbilt University)

8:15 am The integration of microRNAs into a Network Bioinformatics Approach.
Stephen Y. Chan, MD PhD. Boston, MA (Brigham and Women's Hospital)

8:50 am Integration of Gene Networks to Identify Novel Genes Related to Lung Function
Hakon Hakonarson, M.D., Ph.D. Philadelphia, PH (Children's Hospital of Philadelphia)

9:25 am Abstract Award Recipient
Raf/ERK Drives the Proliferative and Invasive Phenotype of BMPR2-silenced Pulmonary Artery Endothelial Cells
Keytam S. Awad, PhD, Bethesda, MD (CCMD NIH)

9:45 am Break (15 min)

10:00 am Effects of Estrogen and Sex on Gene Expression in PAH
Margaret MacLean, PhD. Glasgow, Scotland, UK (University of Glasgow)

10:35 am Phenotypes Matter too: Bioinformatic Strategies to Study (Pediatric) Pulmonary Vascular Diseases
Steven Abman, M.D. Aurora, CO (University of Colorado)

11:10 am Proteomic Profiling of Pediatric Pulmonary Hypertension
Allen Everett, M.D. Baltimore, MD (Hopkins)

12:00 pm Lunch

Session II: The Identification of Rare Variants in Novel Genes that Associate with PAH
Moderator: C. Gregory Elliott, MD, University of Utah

3:00 pm Beyond BMPR2 I: KCNK3, CAV1 and the Use of New Approaches to Provide Alternative Insights into the Pathogenesis of PAH
Wendy K. Chung, MD, PhD. New York, NY (Columbia University)

3:35 pm Splicing and Expression of Wild Type BMPR2 Associates with PAH in BMPR2 Mutation Carriers
Rizwan Hamid, MD, PhD. Nashville, TN (Vanderbilt University)

COURSE SCHEDULE

4:10 pm Rare Variant Discovery in PCH
Hunter Best, PhD. Salt Lake City, UT (ARUP)

4:45 pm Abstract Award Recipient
The Effect of Riociguat in Pulmonary Artery Endothelial Cells Isolated from Patients Undergoing Right Heart Catheterization
Jennifer S. Grant, PhD, Newcastle, UK (Newcastle University)

5:05 pm Rare Variant Discovery in PVOD
Florent Soubrier, Ph.D. Paris, France (INSERM)

6:00 pm Dinner

Friday, September 11, 2015

Session III: The identification of Common Variations in Novel and Known Genes that Associate with PAH
Moderator: Wendy K. Chung, MD

7:00-8:00 am Breakfast

8:05 am Novel Gene Associations with Disease Susceptibility and Severity in Scleroderma Associated PAH
Rachel L. Damico, MD. Baltimore, MD (Johns Hopkins University)

8:40 am Robyn Barst Lecture
Transcriptional High-Throughput Luciferase Reporter Assay Screening and the Discovery of FK506 as a Modifier of BMP Signaling
Edda Spiekerkoetter, MD. Palo Alto, CA (Stanford University)

9:30 am Derivation of Cord Blood Angiogenic Progenitor Cells for the Study and Potential Therapy of Rare Lung Disorders
Christopher D. Baker, MD. Aurora, Colorado (University of Colorado)

10:05 am Break (15 min)

10:20 am Genome-Wide Association Analysis Identifies a Susceptibility Locus for Pulmonary Arterial Hypertension.
Florent Soubrier, Ph.D. Paris, France (INSERM)

10:55 am Meet the Professor Hour: Trainee-Expert Interactive Forums

12:00 pm Lunch

Session IV: Genomic Study Approaches
Moderator: Eric D. Austin, MD MSCI

3:30 pm Biologic Insights in Pulmonary Vascular Disease from an EMR-based Cohort
Evan Brittain, MD, Nashville, TN (Vanderbilt University)

4:05 pm Novel Metrics to Endotype the PH patient
Raed A. Dweik, MD, Cleveland, OH (Cleveland Clinic)

4:40 pm Sample Acquisition and Genotyping: National Biological Sample and Data Repository
William C. Nichols, PhD, Cincinnati, OH (Cincinnati Children's Hospital Medical Center)

5:15 pm Abstract Award Recipient
Loss of Signaling Through the TGF-beta Receptor 3 Causes Male-Predominant Pulmonary Hypertension and Metabolic Dysregulation
Joshua Fessel, MD, PhD, Nashville, TN (Vanderbilt University)

COURSE SCHEDULE

6:00 pm Dinner

8:00 pm After Dinner Talk: Estelle Grover Lecture
The Mitochondria in Pulmonary Vascular Disease
Stephen L. Archer, MD. Ontario, Canada (Queen's University)

Saturday, September 12, 2015

Session V: Epigenetic Mechanisms of Pulmonary Vascular Disease
Moderator: D. Hunter Best, PhD

7:00-8:00 am Breakfast

8:05 am Molecular mechanisms of pulmonary vascular diseases
Soni Savai Pullamsetti, PhD. Bad Nauheim, Germany (Max Planck Institute for Heart and Lung Research)

8:40 am Epigenetic Reprogramming and a Pathologic Phenotype of Lung Fibroblasts
Kurt Stenmark, MD, Aurora, CO (University of Colorado)

9:15 am Epigenetic variations in PAH
Frédéric Perros, Ph.D. (Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie de Québec)

9:50 am Genomic and Functional Consequences of Chronic Hypoxia
Larissa Shimoda, Ph.D. Baltimore, MD (Johns Hopkins University)

10:25 am Break (15 min)

10:40 am Jack Reeves Lecture
Key Cellular and Molecular Programs Regulating Lung Vascular Development
Marlene Rabinovitch, MD. Palo Alto, CA (Stanford University)

12:00 pm Lunch

Session VI: Pulmonary vascular and ventricular dysfunction in the susceptible subject
Moderator: Kenneth Weir, MD

3:00 pm Terry Wagner Lecture
Profiling Genetic and Epigenetic Changes in the Lungs of Patients with Pulmonary Vascular Disease
Micheala A. Aldred Ph.D. Cleveland, OH (Cleveland Clinic)

3:50 pm Variations in Aldostrene synthesis and activity in hypoxia
Bradley Maron, M.D. Boston, MA (Brigham and Women's Hospital)

4:25 pm Novel mechanisms in the pathogenesis of pulmonary hypertension
Jason X.-J. Yuan, MD, PhD. Chicago, IL (University of Arizona)

5:00 pm Gene Transfer of SERCA2a to the Pulmonary Vasculature and Right Ventricle.
Jane A. Leopold, MD. Boston, MA (Brigham and Women's Hospital)

6:00 pm Dinner

7:00 pm Poster Session (evening): Chaired by I.F. McMurtry, C.G. Elliott and K. Stenmark

COURSE SCHEDULE

Sunday, September 13, 2015

Session VII: Translation into Novel Therapeutic Approaches and Monitoring
Moderator: Stephen L. Archer, MD

7:00-8:00 am	Breakfast
8:05 am	Wnt Signalling Irregularities and PAH Vinicio De Jesus Perez, MD, Palo Alto, CA (Stanford University)
8:40 am	Neonatal hyperoxic lung injury may promote a more adaptive RV phenotype later in life Kara N. Goss, MD. Madison, WI (University of Wisconsin)
9:15 am	Novel Insights into Pulmonary Vascular Disease in the Child with Premature-Associated Lung Disease Peter Mourani, MD. Aurora, CO (University of Colorado)
9:50 am	Break (15 min)
10:05 am	A Role for Directed miRNA Antagonism for the Prevention and Treatment of BMPR2 Associated PAH James West, Ph.D. Nashville, TN (Vanderbilt University)
10:40 am	Novel Molecular and Phenotypic Signatures of Pulmonary Vascular Disease in Sickle Cell Lung Disease Roberto F. Machado, MD. Chicago, IL. (University of Illinois at Chicago)
11:15 am	Closing summary (10 min)
12:00 pm	Lunch

The conference will adjourn after lunch.

ABSTRACT PRESENTATIONS

Raf/ERK drives the proliferative and invasive phenotype of BMPR2-silenced pulmonary artery endothelial cells.

Keytam S. Awad,[1] Jason M. Elinoff,[1] Shuibang Wang,[1] Salina Gairhe,[1] Gabriela A. Ferreyra,[1] Rongman Cai,[1] Junfeng Sun,[1] Michael A. Solomon,[1,2] and Robert L. Danner[1]

[1]Critical Care Medicine Department, Clinical Center, and [2]Cardiopulmonary branch, NHLBI, National Institutes of Health, Bethesda, MD, 20892

Rationale: Pulmonary arterial hypertension (PAH) is characterized by a proliferative endothelial cell phenotype, inflammation and pulmonary vascular remodeling. Bone morphogenetic protein type II receptor (BMPR2) loss-of-function has been closely linked to pathologic plexiform lesions with obliteration of distal pulmonary arteries. While disruption of BMP signaling is important, some BMPR2 mutations associated with PAH leave ligand-dependent responses intact. The role of ligand-independent, non-canonical effects of BMPR2 (i.e. interactions with stress kinases) are incompletely understood.

Objective: To examine the phenotypic, transcriptomic and non-canonical signaling consequences of BMPR2 silencing in human pulmonary artery endothelial cells (HPAECs)

Methods and Results: BMPR2 siRNA silencing resulted in a proliferative, pro-migratory HPAEC phenotype and disruption of cytoskeletal architecture. Expression profiling closely reflected these phenotypic changes. Gene set enrichment and promoter analyses, as well as the differential expression of pathway components identified Ras/Raf/ERK/AP1 signaling as an important non-canonical consequence of BMPR2 silencing. Raf family members and ERK1/2 were constitutively activated after BMPR2 knockdown. Gene expression changes associated with BMPR2 silencing overlapped with stress kinase activation by mitogen. Raf inhibitors and low-dose nintedanib, a triple receptor tyrosine kinase (RTK) inhibitor upstream from Ras, reversed the abnormal proliferation and hyper-motility of BMPR2 deficiency.

Conclusions: BMPR2 silencing produces a proliferative, pro-migratory endothelial cell phenotype with disruption of cytoskeletal architecture. Inhibition of dysregulated RTK and stress kinase signaling corrected some of these phenotypic alterations and may be useful in preventing or reversing vascular remodeling in PAH.

Metabolic drivers and sensors of cell proliferation in IPAH

Jarrod W. Barnes[1], Liping Tian[1], Suzy A. A. Comhair[1], Roberto Machado[3], Raed A. Dweik[1,2]. barnesj5@ccf.org.

Departments of Pathobiology[1], Lerner Research Institute and Respiratory Institute[2], Cleveland Clinic, Cleveland, OH, College of Medicine, University of Illinois at Chicago[3].

Background—Metabolic dysregulation has emerged as a major area of research in the pathobiology of idiopathic pulmonary arterial hypertension (IPAH). We recently published that increased O-linked N-acetyl-glucosamine (O-GlcNAc) transferase (OGT) was shown to enhance pulmonary arterial smooth muscle cell (PASMC) proliferation and worsen IPAH disease outcomes. OGT is a nutrient 'sensor' and is involved in cell cycle and signaling, proliferation, and metabolism. Proper homeostasis of the OGT/O-GlcNAc axis is required for proper cell function. An imbalance in the axis can 'drive' disease progression by altering cell proliferation and nutrient metabolism.

Methods—Human IPAH (n=3) and control (n=3) patient PASMCs were subjected to glucose bioenergetics analysis using an extracellular flux analyzer (Seahorse Bioscience) with and without OGT inhibitor. The rates of glycolysis, capacity, and reserve were calculated for these experiments.

Results—Basal glycolytic rates were lower in IPAH PASMCs than controls, but were not significantly different. However, glycolytic capacity and reserve rates were increased in IPAH PASMCs. Upon treatment with an OGT inhibitor, glycolytic capacity and reserve rates in IPAH PASMCs were reduced to control PASMCs levels. Consistent with this, metabolomics studies demonstrated that key tricarboxylic acid cycle intermediates (succinate, α-keto-glutarate, and citrate), that are driven by OXPHOS, increased upon gene-silencing of OGT by siRNA in IPAH PASMCs.

Conclusions—IPAH PASMCs have altered glucose metabolism consistent with the Warburg effect phenomenon. Inhibition of OGT resulted in changes in glucose metabolism similar to control PASMCs. We believe that the OGT/O-GlcNAc axis is a major regulator of the glucose 'metabolic switch' in IPAH PASMCs (similar to cancer cells).

Alveolar macrophage depletion results in worsening of pulmonary hypertension associated with pulmonary fibrosis

Bryant AJ[1], Brown GA[2], Shenoy V[3], and Scott EW[2].

[1]Division of Pulmonary, Critical Care, and Sleep Medicine. [2]Department of Molecular Genetics and Microbiology. [3]Department of Pharmacodynamics. University of Florida College of Medicine, Gainesville, FL. Funding: Gatorade Fund, UF CTSI

Introduction: Pulmonary hypertension (PH) frequently complicates the care of patients with idiopathic pulmonary fibrosis (IPF) (1). Pulmonary macrophages have previously been shown to be necessary in the development of IPF (2). While it is known that macrophages are involved in the pathogenesis of chronic hypoxia-mediated PH (3), it is unknown if macrophages contribute to PH-associated with fibrosis. We hypothesized that depletion of pulmonary macrophages would be protective against development of PH-associated with a model of pulmonary fibrosis.

Methods: Wild type C57BL/6 underwent 100 mcL intraperitoneal injection with clodronate or PBS liposomes every three days, one week prior to and while receiving either 0.018 U/g bleomycin or vehicle twice weekly for 28 days. In a separate experiment, animals received clodronate or PBS liposomes one week prior to and during chronic hypoxia (4 weeks, 10% FiO2) exposure. Invasive pulmonary hemodynamic measurement was measured at time of harvest.

Results: After bleomycin exposure, mice with pulmonary macrophage depletion had markedly elevated right ventricular systolic pressure (RVSP) compared to controls. Similarly, mice exposed to clodronate and hypoxia had elevated RVSP, compared to vehicle normoxic animals.

Conclusions: Pulmonary macrophage depletion results in worsened PH in a pulmonary fibrosis model, and a chronic hypoxia model of disease. Together, these findings represent a novel pathway for development of therapeutics in PH associated with chronic lung disease.

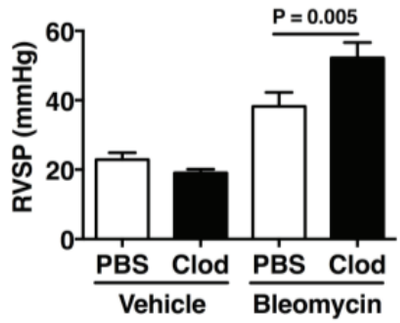

Figure. Right ventricular systolic pressure (RVSP) is increased in mice exposed to bleomycin and clodronate (Clod) versus PBS liposomes.

References:
1. Patel NM, Lederer DJ, Borczuk AC, Kawut SM. Pulmonary hypertension in idiopathic pulmonary fibrosis. Chest 2007; 132: 998-1006.
2. Murray LA, Chen Q, Kramer MS, Hesson DP, Argentieri RL, Peng X, Gulati M, Homer RJ, Russell T, van Rooijen N, Elias JA, Hogaboam CM, Herzog EL. TGF-beta driven lung fibrosis is macrophage dependent and blocked by Serum amyloid P. Int J Biochem Cell Biol 2011; 43: 154-162.
3. Frid MG, Brunetti JA, Burke DL, Carpenter TC, Davie NJ, Reeves JT, Roedersheimer MT, van Rooijen N, Stenmark KR. Hypoxia-induced pulmonary vascular remodeling requires recruitment of circulating mesenchymal precursors of a monocyte/macrophage lineage. The American journal of pathology 2006; 168: 659-669.

Interplay between IL-13 and energy metabolism in pulmonary hypertension

W-K Cho MD, S Rounds MD

Pulmonary/Critical Care, Alpert Medical School of Brown University, Providence, RI

Interleukin-13 (IL-13), a T helper type 2-cell effector cytokine, may play a role in the pathogenesis of pulmonary arterial hypertension (PAH), but the mechanism of this effect is unknown. We reported that IL-13 transgenic mice (IL-13 tg) develop smooth muscle cell (SMC)-driven pulmonary vascular remodeling and pulmonary hypertension (PH) via an IL-13 receptor alpha 2 - Arginase 2 -dependent pathway. Decreased mitochondrial oxidative phosphorylation (decreased glucose oxidation) and increased cytoplasmic glycolysis under aerobic conditions (the Warburg effect) in vascular cells may be important in generation of proliferative phenotype in PAH. Increased fatty acid beta-oxidation (FAO) in vascular cell mitochondria leads to decreased mitochondrial glucose oxidation. This mutually inhibitory balance (the Randle cycle), may also be important in PAH. We observed increased gene expression of enzymes involved in glycolysis and FAO in IL-13 tg lungs. We also found increased lactate and decreased oxygen consumption (Seahorse XR24 Analyzer) in proliferating IL-13-treated human pulmonary artery SMC (hPASMC), compared to non-treated hPASMC. One-month treatment of IL-13 tg mice via drinking water with either Dichloroacetate (0.75g/L, to inhibit pyruvate dehydrogenase kinase and increase glucose oxidation) or with Ranolazine Dihydrochloride (0.12g/L, to inhibit FAO) attenuated pulmonary vascular remodeling. RV remodeling and tissue inflammation in IL-13 tg were decreased only in Ranolazine-treated animals. These findings suggest that IL-13 contributes to development of PH by inducing metabolic reprogramming and inflammation. (KO8HL121183)

Loss of Signaling Through the TGF-beta Receptor 3 Causes Male-Predominant Pulmonary Hypertension and Metabolic Dysregulation

Joshua Fessel[1,4], Daniel DeLaughter[2], Jonathan Seidman[2], James West[1], Eric Austin[3], and Joey Barnett[4]

Departments of [1]Medicine, [3]Pediatrics, and [4]Pharmacology, Vanderbilt University, Nashville, TN

[2]Department of Genetics, Harvard Medical School, Boston, MA

While TGF-beta receptor type 3 (TGFBR3) binds both TGF-beta and BMP ligands to signal through canonical Smad pathways, it also interacts with cytoskeletal regulatory pathways. Based on previous data showing that loss-of-function mutations in BMPR2 result in whole body glucose dysregulation, cytoskeletal disruption, and PAH, we sought to determine whether loss of BMP signaling through TGFBR3 would reproduce a similar phenotype. As homozygous deletion of TGFBR3 is lethal due to the failure of coronary vessel development, we studied male and female TGFBR3 +/- mice and their wild-type littermate controls. Mice were placed on a Western-type diet (60% fat calories) for 12 weeks. Metabolic parameters including energy expenditure, activity, glucose tolerance, and insulin production were quantified. Cardiac output was measured by echocardiography, and RVSP was invasively determined by closed-chest catheterization. We found that male TGFBR3 +/- mice challenged with a Western diet had higher body fat percentage, impaired glucose tolerance, and increased pulmonary vascular resistance driven primarily by a heart failure phenotype. However, female TGFBR3 +/- mice had increased lean muscle mass, increased whole body efficiency of energy utilization (manifest as increased activity without an increase in energy expenditure), preserved glucose tolerance, and preserved pulmonary hemodynamics. Isolated RV myocardial oxygen consumption was decreased in the TGFBR3 +/-, and in cell culture, loss of both copies of TGFBR3 resulted in suppression of PGC1-beta and suppression of expression of electron transport chain genes. Further studies may provide insight into the mechanisms underlying increased severity of PAH in males.

Estrogen Receptor (ER) is Expressed in the Failing Right Ventricle and Mediates Protective Effects of 17β-Estradiol (E2) on Pro-survival Signaling in Stressed Rat Cardiomyoblasts

Andrea Frump[1], Sandra Breuils-Bonnet[2], Thomas Jones[1], Steeve Provencher[2], Sébastien Bonnet[2], Tim Lahm[1]

Indiana University[1], University of Laval[2]

Right ventricular (RV) function is the major determinant of survival in pulmonary arterial hypertension (PAH). Women with PAH exhibit superior RV function and survival compared to men; attributed to E2 cardioprotective effects. Mechanisms of E2-mediated RV protection have not been identified. We hypothesized that E2, in an ER-dependent manner, exerts beneficial effects on cardiomyoblast pro-survival signaling.

Studies were performed in RV tissue from patients with compensated or decompensated RV function, RV cardiomyocytes isolated from rats with sugen/hypoxia (SuHx)-induced RV failure and H9C2 cardiomyoblasts exposed to TNFα (10 ng/ml) or staurosporine (50 nM) for 1, 4 or 8h. Cells were pretreated with E2 (100 nM) in absence or presence of ER-antagonist fulvestrant (100 nM) or siRNA directed against ERα or ERβ. Stress responses (pP38), prosurvival (Bcl2, pERK1/2), proapoptotic (Bax), and procontractile/proangiogenic signaling (apelin) were assessed by western blot. $P<0.05$ was considered significant.

ER was expressed in RVs from patients with RVF and RV cardiomyocytes from SuHx rats. E2 increased pro-survival signaling (increased Bcl2/Bax ratio) and pro-angiogenic and pro-contractile apelin expression in RVs from rats with SuHx-induced RVF ($p<0.05$). TNFα and staurosporine induced H9C2 dysfunction (decreased Bcl2/Bax ratio and apelin; increased p38 and ERK activation; all $p<0.05$ vs untreated control). E2 attenuated TNF-α and staurosporine-induced alterations in Bcl2/Bax, apelin and p38 ($p<0.05$ for all endpoints). E2 protection was abrogated in presence of ER antagonist or ERα siRNA ($p<0.05$).

ERs are expressed in the failing RV and E2, via ERα, enhances pro-survival, pro-angiogenic and pro-contractile signaling in stressed rat cardiomyoblasts, suggesting E2 and ER as mediators of cardiomyocyte protection in RVF.

Loss of Caveolin-1 Induces an Invasive, Proliferative, and Inflammatory Phenotype in Human Pulmonary Artery Endothelial Cells

Salina Gairhe, PhD., Keytam S Awad, PhD., Jason Elinoff, MD and Robert L. Danner, MD

Critical Care Medicine Department, Clinical Center, National Institute of Health, Bethesda, MD.

Background: Caveolin-1 (CAV-1) is an endothelial scaffolding protein located in flask-shaped invaginations present in the plasma membrane. CAV-1 interacts with several key molecules relevant to pulmonary arterial hypertension (PAH) via its scaffolding domain. Recently, a heterozygous single nucleotide deletion resulting in CAV-1 loss-of-function has been linked to the development of PAH. However, the relationship between CAV-1 loss-of-function and the development of the dysfunctional pulmonary endothelial phenotype associated with PAH pathogenesis is unknown. Here, we hypothesized that CAV-1 loss-of-function in the pulmonary artery endothelium leads to a hyperproliferative, invasive and pro-inflammatory phenotype that contributes to the pathogenic pulmonary vascular remodeling seen in patients with PAH.

Methods & Results: In primary, human pulmonary artery endothelial cells (PAECs), CAV-1 was knocked down using an siRNA approach. This produced an efficient ≥80% knockdown of CAV-1 protein. Cells were then examined for proliferation, migration and the expression of inflammatory mediators and markers. Using an MTS assay and ATP production, we found increased cell proliferation in Cav-1-silenced compared to control PAECs. Loss of CAV-1 function also increased PAECs migration. Similarly, the mRNA and protein expression of IL-6, ICAM1 and VCAM1 increased after CAV-1 knockdown.

Conclusion: These findings demonstrate that CAV-1 loss-of-function in PAECs leads to a proliferative, hypermigratory and pro-inflammatory phenotype. Thus, CAV-1 gene silencing may be a useful in vitro model to study molecular mechanisms important to PAH pathogenesis.

Genetic Variations in Resistin Family Genes and Risk of Pulmonary Arterial Hypertension

L. Gao[1], A. Poloczek[1], N.M. Rafaels[1], L. Hummers[1], S.C. Mathai[1], P.M. Hassoun[1], K.C. Barnes[1], R.A. Johns[1]

[1]Johns Hopkins University - Baltimore/US

Rationale: Pulmonary arterial hypertension (PAH) is progressive disease resulting in death from right ventricular (RV) failure and is the leading cause of mortality in patients with scleroderma (SSc). We have found the resistin family of proteins (i.e., Resistin and RELMβ) playing a critical role in PAH and RV failure. We aim to investigate whether genetic variations in resistin family genes and downstream signaling molecules influence susceptibility to PAH.

Methods: In a case-control study, 80 IPAH, 135 SSc-PAH, 357 SSc only patients and 458 controls of European descent were genotyped for 73 tagging single nucleotide polymorphisms (SNPs) in five genes: RETN, RETNLB, EDN1, VEGFA and VEGFR2 or KDR. The Illumina BeadXpress platform was utilized and the association between SNPs and PAH risk was examined using the trend test.

Results: RETNLB promoter SNP (rs6788267) was associated with risk of SSc-PAH (P=0.023) and two additional SNPs (rs11708527, rs9878093) were associated with PCWP (P=0.01) in both IPAH and SSc-PAH patients. Two markers in RETN (rs3745367 and rs1862513) were associated with reduced CI (P=0.034 and 0.044, respectively) among IPAH patients. In addition, two EDN1 SNPs (rs4714384 and rs9296345) provided the most compelling evidence for association with risk of SSc-PAH (P=0.0007 and 0.0002, respectively); another two SNPs in VEGFA were associated with decreased PCWP (P=0.01); and 5 SNPs in VEGFR2 were associated with CI (rs12498529, P=0.001), mPAP (rs1870378, P=0.01), RVSP (rs1870378 and rs1870377, P=0.003 and 0.007, respectively) and PCWP (rs1531290, P=0.008), among the combined PAH patients.

Conclusions: Our findings suggest that polymorphisms in resistin family genes and downstream signaling molecules are significantly associated with the risk and hemodynamic measurements of PAH.

Bioinformatic-based analysis identifies HIPPO dysfunction as a novel trigger of mTOR- Akt activation and proliferation/ apoptosis imbalance of vascular smooth muscle cells in pulmonary arterial hypertension

Dmitry Goncharov[1], Neil Kelly[1], Andressa Pena[1], Kaori Ihida-Stansbury[2], Horace DeLisser[2], Steven Kawut[2], Herman Silljé[3], Steven Shapiro[1], Yutong Zhao[1], Elena Goncharova[1]

[1]University of Pittsburgh School of Medicine, Pittsburgh, PA, [2]University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA, [3]University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Proliferation/apoptosis imbalance of distal pulmonary artery vascular smooth muscle cells (PAVSMC) is a key component of vascular remodeling in pulmonary arterial hypertension (PAH). The database tool MalaCards predicted genes related to growth-suppressor HIPPO pathway as the 2nd most highly related to PAH based upon their overlap within mined disease resources. Protein kinase LATS1, a central component of HIPPO pathway, was inactivated in PAVSM in small remodeled PAs from patients with PAH and rodents with SU5416/hypoxia- induced PH. Deficiency of active P-T1079 LATS1 in distal human PAH PAVSMC was associated with up-regulation of its reciprocal downstream effectors, transcriptional co-activators Yap/Taz, elevated proliferation, reduced apoptosis, and deficiency of pro-apoptotic Bim. A computational protein interactome-based screen revealed that members of the AKT signaling pathway were among the most significantly enriched in the shortest paths between HIPPO and PAH. Using dephospho- and phospho-mimic LATS1 constructs, siRNA- and inhibitor-based approaches, we found that up-regulation of Yap/Taz caused by HIPPO/LATS1 inactivation is required for persistent activation of Akt, its upstream activator mTORC2 and downstream effector mTORC1, and maintenance of proliferative/apoptosis-resistant PAH PAVSMC phenotype. Importantly, LATS1 down-regulation in non-diseased distal human PAVSMC induced Yap/Taz accumulation, up-regulated mTORC2-Akt-mTORC1 axis and increased proliferation and Bim deficiency, re-capitulating PAH PAVSMC phenotype. Collectively, these data demonstrate critical role of HIPPO/LATS1 inactivation in pro-proliferative/apoptosis- resistant PAVSMC phenotype and confirm the attractiveness of a bioinformatic approach in studying the mechanisms of PAH pathogenesis.

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The effect of riociguat in pulmonary artery endothelial cells isolated from patients undergoing right heart catheterisation.

Jennifer S. Grant[1], Marie-Hélène Ruchaud-Sparagano[1], Logan Thirugnanasothy[1], Sasiharan Sithamparanathan[1], Paul A. Corris[1].

(1. Institute of Cellular Medicine, Newcastle University, Newcastle-upon-Tyne, NE2 4HH.)

Idiopathic pulmonary arterial hypertension (iPAH) is a complex disease characterised by narrowing and remodelling of the small pulmonary arteries resulting in an increase in pulmonary artery pressure and right heart failure. This process involves all cell types within the vessel wall with endothelial dysfunction being a principle factor involved in disease progression. Mortality rates remain unacceptably high despite current treatment which predominantly work as vasodilators and specific endothelial effects are not well characterised.

In this study, pulmonary artery endothelial cells (PAECs) were successfully isolated from the balloon tip of the Swan-Ganz catheter from iPAH patients undergoing right heart catheterisation. These primary cells were cultured effectively in vitro and characterised as endothelial cells with high expression levels of CD31 (>99% CD31+) and von Willebrand factor. High levels of CD34 were found at low passage (>96%) with progressive loss of the marker at higher passage (<10% at passage 9). Treatment of these primary PAECs with 10μM riociguat, a soluble guanylate cyclase stimulator, reduced HIF-1α expression induced by hypoxic exposure. This was accompanied by a reduction in vascular endothelial growth factor receptor 2 (VEGFR2) expression.

These findings demonstrate that endothelial cells isolated from the pulmonary vasculature of iPAH patients undergoing right heart catheterisation present an effective tool to study PH drugs in vitro. Furthermore, this is the first time that riociguat is shown to regulate the HIF-1α/VEGF signalling pathway in primary PAECs from iPAH patients, suggesting that soluble guanylate cyclase stimulators may also have an impact on angiogenic pathways in endothelial cells.

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Blood MiRNAs to Monitor Pulmonary Hypertension – Effect of Exercise Training

Gabriele Grunig[1], Serhiy Pylawka[1]*, Ian Ladran[1]*, Christina A. Eichstaedt[2,3] , Christine Fischer[3], Ekkehard Grünig[2]; [1]Mirna Analytics LLC, New York, NY, USA, [2]Thoraxclinic at the University Clinic Heidelberg, Heidelberg, Germany, [3]Institute for Human Genetics, University of Heidelberg, Heidelberg, Germany.*

Supervised exercise training has been shown to be a beneficial and effective add-on to medical therapy in pulmonary arterial hypertension (PAH), of different etiologies (e.g. heritable PAH, or autoimmunity associated PAH). In this study we hypothesized that exercise would induce epigenetic changes, in particular in miRNA profiles, and that these changes could be measured in the blood.

Paired whole blood RNA samples, and paired serum RNA samples were obtained from patients with heritable PAH (n=6 pairs, each sample type) before and after a 3- week rehabilitation with specialized, low dose and closely supervised exercise training. The whole blood RNA samples were analyzed by miRNAseq. Twenty-five miRNA species demonstrated increased or decreased read numbers following exercise. Quantitative real time qPCR was then used to determine the levels of the miRNA species in a new set of paired whole-blood RNA and in paired serum RNA samples. Comparing miRNAseq and qPCR, whole blood and serum RNA, and paired RNA samples, the data demonstrated the technical robustness of the techniques. qPCR confirmed the initial patterns for 12 miRNAs. Further studies are under way to understand the biological role of circulating miRNAs, and to determine the significance of therapeutic exercise on the levels of the circulating miRNAs in PAH patients.

*GG, SP, IL are co-founders of Mirna Analytics LLC (New York).

Title: Increased Sodium-Hydrogen Exchanger Activity Contributes to Pathologic Pulmonary Arterial Smooth Muscle Cell (PASMC) Function in Pulmonary Arterial Hypertension (PAH)

Authors: Huetsch JC[1], Shimoda LA[1]

[1]Division of Pulmonary and Critical Care Medicine, Johns Hopkins School of Medicine, Baltimore, MD

Rationale: Remodeling of the pulmonary vasculature, involving proliferation and migration of PASMCs, is a key feature of pulmonary hypertension. Prior work has shown that the sodium-hydrogen exchanger (NHE1) is necessary for pathologic vascular remodeling in response to chronic hypoxia. We hypothesize that altered NHE1 activity contributes to pathologic PASMC function in PAH.

Methods: Two PAH models were used. Firstly, PASMCs were isolated from Wistar rats exposed to the vascular endothelial growth factor receptor inhibitor SU5416 plus 3 wk of 10% oxygen followed by 2 wk of normoxia (SuHx model), and control rats exposed to vehicle and 5 wk of normoxia. The second model used PASMCs isolated from PAH patients (n=7) and control subjects (n=10) (provided by PHBI). To measure NHE1 activity, PASMCs were incubated with pH-sensitive fluorescent dye and the Na+-dependent recovery of intracellular pH following ammonium pulse challenge was measured via fluorescence microscopy. PASMC proliferation was assessed via BrdU incorporation. PASMC migration was measured using a transwell assay. Ethyl-isopropyl amiloride was used to inhibit NHE1.

Results: In both models, NHE1 protein expression and activity were greater in PAH PASMCs compared to controls. PASMCs isolated from SuHx rats exhibited increased proliferation and migration relative to controls; pharmacologic inhibition of NHE1 significantly blunted these increases. Additionally, NHE1 inhibition decreased proliferation in PASMCs from human PAH patients.

Conclusions: NHE1 expression and activity were increased in two models of PAH. NHE1 inhibition decreased proliferation and migration in PASMCs from PAH models, suggesting NHE1 could be a potential therapeutic target.

PPARγ Activation Attenuates ET-1 Expression and Endothelial Dysfunction by Inhibiting miR-27a in Sickle Cell Mice Lungs with Pulmonary Hypertension and Hemin-Treated HPAECs

Bum-Yong Kang[1], Roy L Sutliff[1], David Archer[2], and C. Michael Hart[1]

[1]Department of Medicine, Atlanta Veterans Affairs and Emory University Medical Centers, Atlanta, GA, [2]Department of Pediatrics, Emory University School of Medicine, Atlanta, GA

Rationale: Pulmonary hypertension (PH), a serious complication of sickle cell disease (SCD), causes significant morbidity and mortality. We recently reported that the PPARγ ligand, rosiglitazone (RSG), attenuates hypoxia-induced PH and increases in microRNA-27a (miR-27a) and endothelin 1 (ET-1) expression.

Objective: To examine these pathways in SCD, we hypothesized that increased levels of miR-27a reduce PPARγ expression leading to increased ET-1 expression, endothelial dysfunction, and SCD-PH.

Methods and Results: Levels of miR-27a, ET-1, and endothelial specific markers (ICAM1, VE-CAD, PECAM1, FLT1, and SELE) were increased in the lungs of 8-10 week old sickle cell mice (SS) compared to littermate control (AA) mice whereas PPARγ levels were reduced. In parallel studies, 8-10 week old SS mice or AA mice were gavaged with RSG (10 mg/kg/d) or vehicle for 10 days. RSG attenuated increases in miR-27a and ET-1 in SS mouse lung. In vitro, human pulmonary artery endothelial cells (HPAECs) were treated with control (DMSO) or hemin (5 M) for 72 hours. Hemin increased miR-27a, ET-1, and endothelial dysfunction, and reduced PPARγ expression. These alterations were attenuated by treatment with RSG (10 μM) during the last 24 hours of hemin treatment. In contrast, inhibition of miR-27a or overexpression of PPARγ restored hemin-induced ET-1 and endothelial dysfunction.

Conclusion: Collectively, these findings suggest that in SCD-PH pathogenesis miR-27a reduces PPARγ thereby increasing ET-1 and endothelial dysfunction and that PPARγ activation may represent a novel therapeutic approach in SCD-PH pathogenesis.

Protective Effects Of Endogenous Sex Hormones On Right Ventricular (RV) Function After Acute Exercise In Su5416/ Hypoxia-Induced Pulmonary Hypertension (SuHx-PH)

T Lahm, A Frump, A Vayl, A Fisher, M Albrecht, T Jones, T Cook, MB Brown.

Indiana University.

Women with pulmonary arterial hypertension exhibit better survival and more preserved RV function than men. The underlying mechanisms are unknown. We hypothesized that female SuHx-PH rats tolerate acute exercise-induced RV stress better than their male counterparts, and that 17beta-estradiol (E2) mediates this effect.

Male and age-matched intact female SuHx-PH rats, as well as ovariectomized (OVX) females with or without concomitant E2 repletion (75 mcg/kg/d) underwent an acute exercise challenge (treadmill running for 45 min at 75% of previously determined VO2max). Immediately post-exercise, RV function was determined echocardiographically, followed by measurement of RV systolic pressure (RVSP). RV eNOS activation, pro-apoptotic signaling and autophagy were determined. $P<0.05$ by ANOVA was considered significant.

Female rats exhibited superior RV function post-exercise (increased velocity time integral, stroke volume and cardiac index by 50-70%; $p<0.05$ for all parameters). OVX worsened exercise capacity, shortened the time to achieve VO2max, increased RVSP, and abrogated favorable RV adaptations. E2 replacement following OVX improved VO2max (by 50%; $p<0.05$) and the time to achieve it, reduced RVSP (by 60%; $p<0.05$), and improved echocardiographic parameters of RV function by 60-80% ($p<0.05$). On a cellular level, OVX decreased eNOS phosphorylation, enhanced pro-apoptotic signaling (decreased bcl-2/bax ratio) and worsened blockage of autophagic flux (increased p62). In turn, E2 treatment of OVX animals enhanced RV eNOS phosphorylation, attenuated pro-apoptotic signaling and improved autophagic flux ($p<0.05$ vs OVX). Cardioprotective effects of E2 were recapitulated in cultured H9C2 cardiomyoblasts.

Female SuHx-PH rats exhibit more favorable RV responses to acute exercise compared to diseased males, mediated by favorable effects of endogenous sex hormones on RV eNOS activation, pro-apoptotic signaling and autophagy.

Enhanced Caveolin-1 Expression in Pulmonary Hypertensive Smooth Muscle Cells despite the Loss of Cavin1

R. Mathew, J. Huang, MH Gewitz

Department of Pediatrics, New York Medical College, Valhalla, NY 10595

Progressive endothelial caveolin-1 loss occurs before the onset of pulmonary hypertension (PH). Extensive loss of endothelial caveolin-1 associated with enhanced caveolin-1 expression in smooth muscle cells (SMC) has been reported in human PH and in the monocrotaline (M) model. Cavin1 participates in caveolae formation and incorporates caveolin-1 into caveolae. Caveolin-2 requires caveolin-1 for its localization in caveolae. To examine the alterations in caveolin-1 and related factors, rats were injected with M (40 mg/kg), and another group with M injection was exposed to hypoxia (M+H). At 4 weeks, hemodynamic data, expression of caveolin-1, caveolin-2 and cavin1 were examined, and compared with the controls (C). PH and right ventricular hypertrophy were observed in the M group with a further increase in the M+H group. The total caveolin-1 expression in the lungs of the M group was significantly reduced compared with C (17±4% vs 100%, $P<0.05$), but it was increased in the M+H group (81±4% vs M, 17±4%, $P<0.05$). In controls, 100% of the arteries exhibited robust expression of endothelial caveolin-1, without enhanced caveolin-1 expression in SMC. In the M and M+H groups, endothelial caveolin-1 was observed only in 13±1% and 8±1% of arteries respectively, but 22±0.5% and 61±2% revealed enhanced expression of caveolin-1 in SMC. The expression of cavin1 (M, 7±4%, M+H, 7±3% vs C, 100%) and caveolin-2 (M, 30±4%, M+H, 26±5% vs C, 100%) was significantly reduced in M and M+H groups. We conclude that: a) the loss of cavin1 and caveolin-2 in M and M+H groups indicates the loss of caveolae, and b) the SMC caveolin-1 may be in non-caveolar region, and may facilitate PH progression.

Effect of 2-aminoetildiphenylborinate treatment on pulmonary artery function and structure from neonatal lambs with pulmonary hypertension induced by chronic hypoxia.

Castillo-Galán, S[1]., Quezada, S[1]., Beñaldo, F[1]., Ebensperger, R[1]., Ebensperger, G[1]., Herrera, E[1, 2]., Llanos, A[1, 2]., Reyes, R[1].

[1]Programa de Fisiopatología, ICBM, Facultad de Medicina; [2]International Center for Andean Studies (INCAS); Universidad de Chile, Chile. vicreyes@med.uchile.cl.

Neonatal pulmonary hypertension (NPH) may result from perinatal chronic hypoxia. Ex vivo evidence indicates that store operated channels (SOC) are involved in the regulation of the pulmonary artery reactivity and remodeling, particularly in response to hypoxia. We have previously shown that a single dose 2-aminoethyldiphenylborinate (2-APB) blocks SOC and reduces hypoxic pulmonary vasoconstriction in newborn lambs. We hypothesize that a treatment with repeated doses of 2-APB will reduce the pulmonary hypertension, and the pulmonary artery hyperreactivity and pathologic remodeling in lambs with NPH induced by perinatal chronic hypoxia.

Newborn lambs with partial gestation at high altitude (3600m) and returned to lowlands 2 days after delivery were instrumented and subjected to a ten-day treatment with 2-APB (n=5) or its vehicle (n=5). Basal cardiovascular variables were monitored daily, the response to an acute hypoxic challenge was tested one day after the end of the treatment, and euthanasia for ex vivo and in vitro experiments ocurred 2 days after the end of the treatment.

Newborns treated with 2-APB showed a reduction in the mPAP and a cardiac output (CO), compared to control. Reduced hyperreactivity to hypoxia, tromboxane and endothelin-1, and increased relaxation to 8-BrcGMP were also observed. These animals exhibited a decrease in the pulmonary artery medial layer thickening and α-actin expression. Pulmonary VEGF-A transcripts were also reduced.

This set of results suggests that 2-APB partially reverses NPH, despite its partial effects on the cardiac function.

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Changes in Protein Ubiquitination in Hypoxia-induced Pulmonary Hypertension

Brandy E. Wade, C. Michael Hart, Roy L. Sutliff*

Division of Pulmonary, Allergy and Critical Care Medicine, Atlanta VA Medical Center/ Emory University, Atlanta, GA

Pulmonary Hypertension (PH) is characterized by the sustained increase in pulmonary arterial pressure. Increased pulmonary vascular pressure and resistance result in right ventricle (RV) hypertrophy and can ultimately lead to RV failure and death. The pathogenesis of vascular cell proliferation and vascular remodeling in PH is incompletely defined. Current therapeutic approaches employ vasodilators but do not address the cellular proliferation and vascular remodeling that underlie the pathogenesis of PH. To more directly address mechanisms of cell proliferation in PH, this research focuses on the ubiquitin proteasome system (UPS) which plays a critical role in cellular homeostasis by regulating protein stability. Current evidence demonstrates changes in UPS activity in PH; however, the proteins and pathways impacted are poorly defined. To identify proteins with altered ubiquitination in response to hypoxia exposure, mice were exposed to normoxic or hypoxic conditions for 3 weeks. Lungs were harvested and the PTMScan® Ubiquitin Remnant Motif (K-ε-GG) Kit was used to precipitate ubiquitinated proteins for analysis by mass spectrometry (MS). 243 proteins were identified with a fold change ≥±2. In silico analysis identified proteins that promote cell proliferation. Tax1bp1, Hspa8, Hspb1, and Fhl1 interact with important cellular proliferation pathways and exhibit decreased ubiquitination in lungs from mice exposed to hypoxia, suggesting an increase in their stability in hypoxia. These results using MS and in silico analysis suggest that hypoxia-induced changes in the UPS may impact proteins involved in pathways that regulate cellular proliferation by increasing or decreasing their stability.

High altitude Gestation and Prenatal Programming of Maxi-K Channel Related Pulmonary Arterial Vasorelaxation in Fetal and Newborn Lamb

Carla Blum-Johnston, Richard Thorpe, Alexander Brunelle, Chelsea Wee, Quintin Blood, Monica R Romero, Lawrence D Longo, William J Pearce, David Hessinger, Sean M Wilson

Center for Perinatal Biology, Advanced Imaging and Microscopy Core, Loma Linda University School of Medicine, Loma Linda CA

Previous evidence suggests high altitude (HA) long-term intrauterine hypoxia causes the lungs of fetal and newborn lambs to develop pathologies consistent with pulmonary hypertension. In this investigation, we hypothesized that HA gestation impairs bradykinin (BK) induced vessel relaxation in newborns by impinging on large conductance calcium-activated potassium channel (Maxi-K) dependent vasorelaxation. This was evaluated by performing myography, Western Immunoblot, flow cytometry, and patch voltage clamp on pulmonary arteries and myocytes isolated from term fetuses and 2-week old newborn lambs housed at low altitude (LA; 335m) or HA (3801 m >100 days). The relaxation responses to BK were partially blocked by the Maxi-K channel inhibitor 1 mM Tetraethylammonium in HA fetal and LA newborn vessels, but not other groups. In fetus, HA increased Maxi-K currents. However, Maxi-K alpha subunit protein expression was greatest in whole cell lysates of LA fetus and reduced in HA fetus or after birth. Beta-1 subunit expression increased slightly after birth, but was unaffected by HA. In comparison, HA decreased membrane expression of beta-1 expression relative to alpha in the fetus. The distinct influences of HA on function compared to expression in the fetus and newborn suggest alterations in Maxi-K channel translocation to the plasma membrane may be involved. Overall, HA gestation accelerates BK mediated pulmonary arterial vasorelaxation in preparation for birth in a rarified environment. However, postnatal development of vasorelaxation is stalled, increasing susceptibility to the development of pulmonary hypertension (NIH and NSF).

PARTICIPANTS

** denotes conference speaker*

Steven Abman, MD*
University of Colorado
Childrens Hospital Colorado
1717 E. Arizona Avenue
Denver, CO 80210
Tel: 720-777-5821
steven.abman@ucdenver.edu

Micheala Aldred, PhD*
Cleveland Clinic
Genomic Med Institute
9500 Euclid Ave NE-50
Cleveland, OH 44195
Tel: 216-445-4336
aldredm@ccf.org

Stephen Archer, MD*
Queen's University
94 Stuart St. Etherington Hall 3041
Kingston, ON K7L3N6
Tel: 6135336327
archers@queensu.ca

Maziar Assadi Gehr, MD
Actelion Pharmaceuticals Ltd.
Gewerbestrasse 16, H95-03-B.11
Allschwil, Switzerland 4123
Tel: 41-79-4408432
maziar.assadi-gehr@actelion.com

Eric Austin, MD, MSCI*
Vanderbilt University
Med Center North
MCN DD-2205
2201 West End Ave
Nashville, TN 37235
Tel: 615-343-7617
eric.austin@vanderbilt.edu

Keytam Awad, PhD
CCMD, NIH
10 Center Drive, Bldg 10 Rm 4D14
Bethesda, MD, 20892
Tel: (330) 261-1715
awadks@cc.nih.gov

Christopher Baker, MD*
The Children's Hospital
Pediatrics Heart Lung Center
12800 E 19th Ave Mailstop 8317
Aurora, CO 80045
Tel: 720-777-6181
christopher.baker@ucdenver.edu

Jarrod Barnes, PhD
Cleveland Clinic
Lerner Research Institute
9500 Euclid Ave,
Cleveland, OH 44195
Tel: 706-713-0175
barnesj50@ccf.org

Hunter Best, PhD*
University of Utah School of Medicine
30 N 1900 E,
Salt Lake City, UT 84132
Tel: 801-583-2787 x2526
hunter.best@utah.edu

Stephen Black, PhD
University of Arizona
1501 N. Campbell Ave, Room 6334
Tucson, AZ 85719
Tel: 706-910-4295
steveblack@email.arizona.edu

Evan Brittain, MD, MSc*
Vanderbilt University
2201 West End Ave,
Nashville, TN 37235
Tel: 615-975-2627
evan.brittain@vanderbilt.edu

Andrew Bryant, MD
University of Florida College of Medicine
1304 NW 121st Way
Gainesville, FL 32606-5274
United States
Tel: 336-471-5703
andrew.bryant@medicine.ufl.edu

Stephen Chan, MD, PhD*
Brigham and Women's Hospital
75 Francis St,
Boston, MA 02115
Tel: 857-222-9055
stephen.y.chan@gmail.com

Li-Yuan Chen, PhD
NIH CCMD
9000 Rockville Pike
Bethesda, MD 20841
Tel: 301-922-3035
Chenl2@mail.nih.gov

Won -Kyung Cho, MD
Brown University
Warren Alpert Medical School
222 Richmond St,
Providence, RI 02903
Tel: 314-359-9971
won-kyung_cho@brown.edu

Gaurav Choudhary, MD
Providence VAMC / Brown University
830 Chalkstone Ave, Research Building
Providence, RI 02908
Tel: 401-273-7100 ext.2029
gaurav_choudhary@brown.edu

Wendy Chung, MD, PhD*
Columbia University
116th St & Broadway,
New York, NY 10027
Tel: 212-851-5313
wkc15@columbia.edu

Rachel Damico, MD, PhD*
John Hopkins University
5501 Hopkins Bayview Cir
Baltimore, MD 21224
Tel: 410-614-6311
rdamico1@jhmi.edu

Vinicio De Jesus Perez, MD*
Stanford University
450 Serra Mall,
Stanford, CA 94305
Tel: 650-723-0318
vdejesus@stanford.edu

Raed Dweik, MD*
Cleveland Clinic
Dept of Pulm Allergy & Crit Care Med
9500 Euclid Ave Desk A90
Cleveland, OH 44195
Tel: 216-445-5763
dweikr@ccf.com

Jason Elinoff, MD
CCMD, NIH
10 Center Drive,
Bethesda, MD, 20892
Tel: 301-978-6468
elinoffj@cc.nih.gov

C. Gregory Elliot, MD*
Intermountain Medical Center
Dept. of Medicine, 5121 S. Cottonwood #307
Murray, UT 84107
Tel: 801-507-3373
greg.elliot@imail.org

Allen Everett, MD*
John Hopkins University
5501 Hopkins Bayview Cir
Baltimore, MD 21224
Tel: 410-955-5987
aeveret3@jhmi.edu

Josh Fessel, MD, PhD
Vanderbilt University
2201 West End Ave,
Nashville, TN 37235
Tel: 615-513-2761
Fax: 615-343-3480
joshua.p.fessel@vanderbilt.edu

Andrea Frump, PhD
Indiana University School of Medicine
340 W 10th St #6200,
Indianapolis, IN 46202
Tel: 615-483-3657
anfrump@iu.edu

Salina Gairhe, PhD
CCMD,CC, NIH
Building 10, Room 4D05, 9000 Rockville Pike
Bethesda, MD, 20892
Tel: (251)776-2806
salina.gairhe@nih.gov

Elena Goncharova, PhD
University of Pittsburgh
BST E1259 200 Lothrop Street
Pittsburgh, PA 15261
Tel: 215-756-4563
eag59@pitt.edu

Ryan Good, MD
University of Colorado
13121 E 17th Avenue, MS 8414
Aurora, CO 80045
Tel: 585-576-7577
ryan.good@childrenscolorado.org

Kara Goss, MD*
University of Wisconsin
Madison, WI 53706
Tel: 317-220-9070
kngoss@medicine.wisc.edu

Jennifer Grant, PhD
Newcastle University
Rm. M3.056 William Leech Building
Framlington Place
Newcastle-Upon-Tyne, UK NE2 4HH
Tel: 44-0751-835-9879
jenny.grant@newcastle.ac.uk

Eva Grayck, MD
University of Colorado
Anschutz Medical Campus
12700 E. 19th Ave, B131
Aurora, CO 80045
Tel: 303-724-5615
eva.grayck@ucdenver.edu

Gabriele Grunig, DVM, PhD
NYU Medical Center
152 The Knoll
Syosset, NY, 11791
Tel: 516-713-7754
ggrunig1@earthlink.net

Hakon Hakonarson, MD, PhD*
The Children's Hospital of Philadelphia
3615 Civic Center Boulevard, Suite 1216/ARC
Philadelphia, PA 19104
Tel: 267-426-0088
hakonarson@email.chop.edu

Rizwan Hamid, MD, PhD*
Vanderbilt University
Med Center North
MCN DD-2205
Nashville, TN 37232-2578
Tel: 615-343-2366
rizwan.hamid@vanderbilt.edu

Rachel Hopper, MD
The Children's Hospital of Philadelphia
3401 Civic Center Blvd. Room 8NW 90
Philadelphia, PA 19104
Tel: 267-398-3637
hopper@email.chop.edu

John Huetsch, MD
John Hopkins University
5501 Hopkins Bayview Cir
Baltimore, MD 21224
Tel: 314-496-9561
jhuetsc1@jhmi.edu

Bum-Yong Kang, PhD
Emory University
Atlanta VA Medical Center
1670 Clairmont Road Room 12C-173
Mailstop 151-P
Decatur, GA 30033
Tel: 404-353-3338
Bum-yong.kang@emory.edu

Tim Lahm, MD
Indiana University
7237 Dover Drive
Indianapolis, IN 46250
Tel: 317-278-0413
tlahm@iu.edu

Jane Leopold, MD*
Brigham and Women's Hospital
Brigham and Women's Hospital
75 Francis St,
Boston, MA 02115
Tel: (617) 525-4846
jleopold@partners.org

Roberto Machado, MD*
University of Illinois Chicago
840 S Wood St, 920-N CSB, MC719
Chicago, IL 60612
Tel: 312-355-5894
machador@uic.edu

Mandy MacLean, PhD*
University of Glasgow
Glasgow G12 8QQ, UK
Tel: 44-141-3304768
mandy.maclean@glasgow.ac.uk

Bradley Maron, MD*
Brigham and Women's Hospital
75 Francis St,
Boston, MA 02115
Tel: 617-525-4857
Fax: 617-525-4830
bmaron@partners.org

Rajamma Mathew, MD
New York Medical College
Department of Pediatrics
40 Sunshine Cottage Rd,
Valhalla, NY 10595
Tel: 646-226-6410
rajamma_mathew@nymc.edu

Ivan McMurtry, PhD*
University of South Alabama
307 N University Blvd,
Mobile, AL 36688
Tel: 251-414-80038
ifmcmurtry@southalabama.edu

Peter Mourani, MD*
University of Colorado School of Medicine
13121 E 17th Avenue, MS 8414
Aurora, CO 80045
Tel: 303-724-2393
peter.mourani@childrenscolorado.org

William Nichols, PhD*
Cincinnati Children's Hospital Medical Center
3333 Burnet Ave, ML 7016, 1469 R
Cincinnati, OH 45229
Tel: 513-284-5948
Bill.nichols#cchmc.org

William Oldham, MD, PhD
Brigham and Women's Hospital
75 Francis St,
Boston, MA 02115Tel: 617-525-4834
Fax: 617-525-4830
woldham@partners.org

Michael Pauciulo, MBA
Cincinnati Children's Hospital Medical Center
3333 Burnet Ave, ML 7016, 1469 R
Cincinnati, OH 45229
Tel: 513-319-7297
mike.pauciulo@cchmc.org

Frédéric Perros, PhD*
IUCPQ
Centre de recherché de UCPQ
Chemin Sainte-Foy
Quebec City, Quebec G1V 4G5
Tel: 418-656-8711 x5813
frederic.perros@gmail.com

Katherine Pratte, MSPH
University of Colorado
Anschutz Medical Campus
12700 E. 19th Ave, B131
Aurora, CO 80045
Tel: 970-402-8198
katherine.pratte@ucdenver.edu

Soni Savai Pullamsetti, PhD*
Max- Plank Institute for Heart and Lung Research
Park Strasse 1, Bad Nauheim
Hessen, Germany, 61231
Tel: (49) 603-270-5380
soni.pullamsetti@mpi_bnimpg.de

Marlene Rabinovitch, MD*
Stanford University
269 Campus Dr. CCOR 1215
Stanford, CA 94305
Tel: 650-723-8239
marlener@stanford.edu

Victor Roberto Reyes, PhD
Universidad de Chile
Av. Salvador 486- Providencia
Santiago RM, Chile 7500922
Tel: 56-9-9050099
virreyc@gmail.com

Sharon Rounds, MD
Brown University
Alpert Medical School
222 Richmond St,
Providence, RI 02903
Tel: 401-226-5741
sharron_rounds@brown.edu

Alison Santana, M.D
University of Colorado
Children's Hospital Colorado
13121 E. 17th Ave, MS 8414
Aurora, CO 80045
Tel: 408-568-6245
alison.santana@childrenscolorado.org

Larissa Shimoda, PhD*
Johns Hopkins University
5501 Hopkins Bayview Circle
Baltimore, MD 21224
Tel: 410-550-5355
shimodal@welch.jhu.edu

Florent Soubrier, MD, PhD*
Universite Pierre et Marie Curie
UMRS 1166 Faculte de medicine
91 bvd de l'hopital
Paris, France 75634
Tel: 33-14-0779743
florent.soubrier@upmc.fr

Edda Spierkeroetter, MD*
Stanford University
450 Serra Mall,
Stanford, CA 94305
Tel: 650-724-1493
eddas@stanford.edu

Kurt Stenmark, MD*
University of Colorado
B131, 12700 E. 19th Avenue
Aurora, CO 80045
Tel: (303) 724-5620
kurt.stenmark@ucdenver.edu

Karthik Suresh, MD
John Hopkins University
5501 Hopkins Bayview Cir
Baltimore, MD 21224
Tel: 502-821-6086
ksuresh2@jhmi.edu

Roy Sutliff, PhD
Emory University
Atlanta VA Medical Center
1670 Clairmont Road Room 12C-173
Mailstop 151-P
Decatur, GA 30033
Tel: 770-634-4016
rsutlif@emory.edu

Thenappan Thenappan, MD
University of Minnesota
3 Morrill Hall
100 Church St. S.E.
Minneapolis MN 55455
Tel: 224-558-7320
tthenapp@umn.edu

Scott Visovatti, MD
University of Michigan
500 S State St,
Ann Arbor, MI 48109
Tel: 734-657-6917
shv@med.umich.edu

Norbert Voelkel, MD*
Virginia Commonwealth University
2607 E. Grace Street
Richmond, VA 23223
Tel: 804-6289614
nvoelkel@mcvh-vcu.edu

Karl Vollmers, PhD
Aria CV, Inc.
1000 Westgate Drive, Suite 150
St. Paul, MN 55114
Tel: 612-876-5241
karlvollmers@gmail.com

Brandy Wade, PhD
Emory University
Atlanta VA Medical Center
1670 Clairmont Road Room 12C-101
Mailstop 151-P
Decatur, GA 30033
Tel: 214-497-5917
bewade@emory.edu

E. Kenneth Weir, MD*
University of Minnesota
3 Morrill Hall
100 Church St. S.E.
Minneapolis MN 55455Tel: 763-473-3226
weirx002@umn.edu

James West, PhD*
Vanderbilt University
2201 West End Ave,
Nashville, TN 37235Tel: 615-343-0895
j.west@vanderbilt.edu

Sean Wilson, PhD
Loma Linda University of Medicine
Center for Perinatal Biology
24851 Circle Dr,
Loma Linda, CA 92354
Tel: 909-647-7021
seanwilson@llu.edu

Jason Yuan, MD, PhD*
University of Arizona Health Sciences Center
1295 Noah Martin Ave PoBox 210202
Tuscon, AZ 85721
Tel: 312-355-5911
jxyuan@uic.edu

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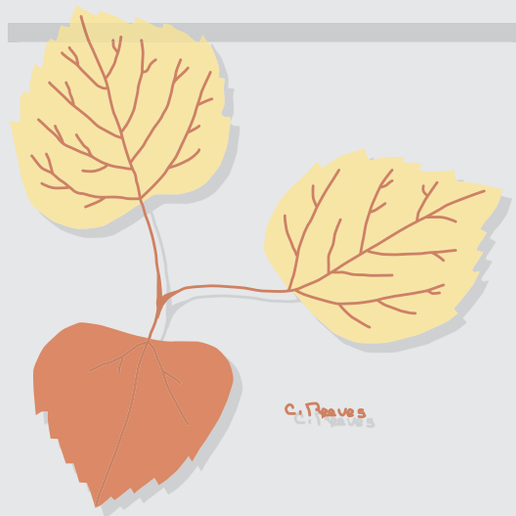

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AMERICAN THORACIC SOCIETY
25 Broadway, 18th Floor, New York, NY 10004
T. 212-315-8600 F. 212-315-6498
thoracic.org