ATS 2025 Grover Conference

PROGRAM

2025 Grover Conference

On Vascular Aging and Regeneration in Pulmonary Hypertension

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The American Thoracic Society and the conference organizing committee gratefully acknowledge the support of this conference by:

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2025 GROVER CONFERENCE
On Vascular Aging and Regeneration In Pulmonary Hypertension

THE PROGRAM

About the Program

Since its inauguration in 1984, the 2025 Grover Conference will be the 21st 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 year's Grover Conference will provide an understanding of how aging impacts the cardio-pulmonary vascular structure and function (via changes in cell function, immune regulation etc.). This understanding is essential for establishing a foundation for diagnoses, therapy/treatment and rescue of pulmonary hypertension alone and in combination with chronic lung diseases.

Learning Objectives

At the conclusion of this program, the learner will be able to:

- Integrate Strategies to Address Vascular Aging in Clinical Practice
  At the conclusion of this session, the learner will be able to apply knowledge of vascular aging mechanisms to develop or refine strategies for diagnosing and managing age-related pulmonary vascular diseases, improving patient outcomes through tailored therapeutic approaches.

- Implement Regenerative Approaches in Pulmonary Hypertension Care
  The learner will be able to evaluate and incorporate emerging regenerative concepts into their practice, enhancing their ability to recognize endothelial dysfunction better and promote vascular repair in patients with pulmonary hypertension.
- Develop Multidisciplinary Interventions for Pulmonary Vascular Diseases
  The learner will be able to design and participate in collaborative, multidisciplinary interventions that integrate research insights into practice, leading to improved care delivery and health outcomes for populations affected by pulmonary vascular diseases.

Who Should Attend

- Clinicians and Researchers: Medical professionals and scientists with expertise in pulmonary hypertension, vascular biology, and lung diseases, particularly those focusing on how aging and regeneration impact pulmonary vascular health.
- Postdoctoral Fellows and Early-Career Scientists: Trainees and early-stage investigators seeking opportunities for mentorship, networking, and advancing their understanding of the most up-to-date topics in pulmonary vascular research.
- International and Interdisciplinary Experts: Professionals from diverse geographic locations and scientific disciplines, including those studying endothelial biology, aging, regeneration, and chronic lung diseases.
- Academic and Industry Stakeholders: Faculty members, researchers, and representatives from biotechnology and pharmaceutical companies interested in advancing treatments and understanding the mechanisms of pulmonary vascular diseases.
- Collaborative Networks: Established investigators and emerging leaders aiming to foster multidisciplinary collaborations and share innovative research approaches in vascular aging and regeneration.

COMMITTEE, SPEAKERS & SESSION CHAIRS

Program Committee

Harry Karmouty-Quintana, PhD
Vladimir Kalinichenko, MD, PhD
Grazyna Kwapiszewska, PhD

Speakers and Session Chairs

Andrew J. Bryant, MD
Stephen Y. Chan, MD, PhD
Laszlo Farkas, MD
Andrea Frump, PhD
Elena Goncharova, PhD
Brian Graham, MD
Sue Gu, MD
Christophe Guignabert, PhD
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ATS 2026

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2025 GROVER CONFERENCE
On Vascular Aging and Regeneration in Pulmonary Hypertension

COURSE SCHEDULE

Wednesday, September 3, 2025

12:00 P.M. - ARRIVALS

6:00 p.m. **Welcome Reception and Dinner**
Harry Karmouty-Quintana, PhD
Grazyna Kwapiszewska, PhD
Vladimir Kalinichenko, MD, PhD

7:00 p.m. **Keynote Address: Vascular Aging in Pulmonary Hypertension: Emerging Therapies and New Frontiers**
Stephen Y. Chan, MD, PhD, University of Pittsburgh

Thursday, September 4, 2025 – Morning Sessions

SESSION I: Vascular Cells Throughout the Human Lifespan

Moderators:
Andrew Bryant, MD (University of Florida)
and Aleksandra Babicheva, PhD (University of Minnesota)

7:00-8:00 A.M. - BREAKFAST

8:05 a.m. **Welcome and Introduction**
Harry Karmouty-Quintana, PhD, University of Texas Health Science Center at Houston
Grazyna Kwapiszewska, PhD, Medical University of Graz
Vladimir Kalinichenko, MD, PhD, University of Arizona

8:15 a.m. **Terry Wagner Memorial Lecture: Impact of Age on Endothelial Heterogeneity in the Pulmonary Vasculature**
Troy Stevens, PhD, University of South Alabama

8:45 a.m. **Abstract Presentation: The Two Faces of B-Catenin in the Distal Lung Endothelium: Homeostasis, Injury and Regeneration**
Courtney A. Stockman, PhD, Cincinnati Children's Hospital Medical Center

9:00 a.m. **Endothelial Cells in Lung Development**
Krithika Lingappan, MD, University of Pennsylvania

Thursday, September 4, 2025 – Morning Sessions

9:30 a.m. **Novel Insights into Endothelial Turnover and Repair in PAH**
Wolfgang Kubler, MD, Charité - University Medicine Berlin, Institute of Physiology

10:00 a.m. **The Aging Endothelial Cell in Lung Disease**
Giovanni Ligresti, PhD, Boston University

10:30 A.M. - BREAK (15 MIN)

10:45 a.m. **Transcriptional Regulation of Lung Endothelial Cells in Vascular Aging and Lung Fibrosis**
Tanya Kalin, MD, PhD, University of Arizona

11:15 a.m. **Targeting the Senescent Endothelium, A Question of Timing?**
Duncan Stewart, MD, Ottawa Hospital Research Institute

11:45 a.m. **Regional Differences in the Distal and Proximal Pulmonary Artery, Implications for the Aging Vasculature**
Lavannya Pandit, MD, PhD, Baylor College of Medicine

12:30 P.M. - LUNCH

Thursday, September 4, 2025 - Optional Lunch & Learn - 1:30 – 2:30 P.M. Registration Required

CAREER DEVELOPMENT 1:

How to Keep Reviewer 2 Engaged, Tips for Grant Writing

Moderators:
Vladimir Kalinichenko, MD, PhD (University of Arizona), Corey Ventetuolo, MD (Brown University) and Elena Goncharova, PhD (University of California, Davies)

Thursday, September 4, 2025 – Afternoon Sessions

SESSION II: Endothelial Dynamics in Health, Disease and Regeneration

Moderators:
Nunzia Caporarello, PhD (Loyola University Chicago)
and Daniels Konja, PhD (National Institutes of Health)

3:00 p.m. **Endothelial Fates in Health and Disease**
Terren Niethamer, PhD, National Cancer Institute

3:30 p.m. **Mechanistic Conflation of Pulmonary Vascular Disease and COPD/Emphysema: Value of Experimental Models**
Enid Neptune MD, ATSF, Johns Hopkins School of Medicine

4:00 p.m. **Endothelial Cell Aging and Lung Regeneration**
Youyang Zhao PhD, Northwestern University Feinberg School of Medicine

Thursday, September 4, 2025 – Afternoon Sessions

4:30 P.M. - BREAK (15 MIN)

4:45 p.m. **Estelle B. Grover Memorial Lecture: Aging Impacts the Heterogeneity of Adult Pulmonary Vascular Progenitors & Angiogenesis in CLD**
Susan Majka, PhD, National Jewish Health

5:15 p.m. **Targeting Endothelial Transcription Factors in Neonatal Pulmonary Hypertension**
Vladimir Kalinichenko, MD, PhD, University of Arizona

5:45 p.m. **Abstract Presentation:**
The Role of Endothelial TBX3 in the Pathogenesis of Pulmonary Arterial Hypertension
Alexandra C. Racanelli, MD, PhD, Albert Einstein College of Medicine

6:00 p.m. **Abstract Presentation:**
Targeting 14-3-3 Protein Mediated Endothelial-Pericyte Crosstalk as a Therapeutic Strategy in Pulmonary Hypertension
Joel James, PhD, Indiana University

6:30 P.M. - DINNER

Friday, September 5, 2025 – Morning Sessions

SESSION III: Pulmonary Hypertension in Aging-Related Lung Diseases

Moderators:
Ke Yuan, PhD (Harvard University)
and Christine Farrell, MD (University of Colorado)

7:00-8:00 A.M. - BREAKFAST

8:05 a.m. **John T. Reeves Memorial Lecture: Oxidative Stress, Mitochondrial Dysregulation, and the Challenge of Vascular Aging**
Norbert F. Voelkel, MD, Amsterdam Medical Center, The Netherlands

8:35 a.m. **United Therapeutics: Treprostinil and ILD**
Franck F. Rahaghi, MD, MHS, Executive Director, Cardiopulmonary Global Medical Affairs

9:05 a.m. **Age-Associated Challenges in Pulmonary Veno-Occlusive Disease**
Akiko Hata, PhD, University of California San Francisco

9:35 a.m. **Sugar-Coated Vessels: How Hyaluronan Promotes Vascular Dysfunction in PH**
Harry Karmouty-Quintana, PhD, University of Texas Health Science Center at Houston

10:05 a.m. **Abstract Presentation:**
Association of RV Endocardial Radiomics with Age in Patients with Pulmonary Hypertension
Rebecca Vanderpool, PhD, The Ohio State University

Friday, September 5, 2025 – Morning Sessions

10:20 A.M. - BREAK (20 MIN)

10:40 a.m. **Breakdown of the Endothelial Barrier Impacts the Development of Fibrosis**
Rachel Knipe, MD, Harvard Medical School

11:10 a.m. **Abstract Presentation:**
Determining Distinct Pulmonary Artery Endothelial Cell Phenotypes in PAH and COPD-PH
Karina M. Massad, MD, National Jewish Health

11:25 a.m. **The Extracellular Matrix in Group 3 Pulmonary Hypertension: Mechanisms and Implications**
Yang Zhou, PhD, Brown University

11:55 a.m. **Abstract Presentation:**
CD301b⁺ cDC2s, but not Batf3⁺ cDC1s, Mediate Hypoxia-Induced Pulmonary Hypertension Via Subset-Specific Activation Programs
Claudia Mickael, PhD, University of Colorado

12:30 P.M. - LUNCH

Friday, September 5, 2025 – Afternoon Sessions

SESSION IV: Intercellular Crosstalk in Vascular Aging

Moderators:
Anne-Karina Perl, PhD (Cincinnati Children's Hospital Medical Center)
and Kel Vin Woo, MD, PhD (Washington University, St. Louis)

3:00 p.m. **Pericytes Function Along the Vascular Tree: From Oxygen Sensing to Inflammation**
Natascha Sommer, MD, Justus Liebig University Giessen

3:30 p.m. **Smooth Muscle Cell in Vascular Remodeling in SSc-PAH**
Markella Ponticos, MD, University College London

4:00 p.m. **Immune Mechanisms of Occlusive Vascular Remodeling**
Maya Kumar, PhD, Stanford University

4:30 P.M. - BREAK (15 MIN)

4:45 p.m. **Vasculature in Interstitial Lung Diseases: Endothelial Cells in Focus**
Grazyna Kwapiszewska, PhD, Medical University of Graz

5:15 p.m. **Robyn J. Barst Memorial Lecture: The Aging Pulmonary Vasculature: Intersection of Inflammation and Injury in CLD**
Sharon I.S. Rounds, MD, ATSF, Brown University

5:45 p.m. **Sex and Age Differences in the Right Ventricle, Additional Hurdles to Overcome?**
Su Gu, MD, University of Colorado, Anschutz Medical Campus

Friday, September 5, 2025 – Afternoon Sessions

6:30 P.M. - DINNER

Saturday, September 6, 2025 – Morning Sessions

SESSION V: Aging, Cell Interactions and Chromatin Remodeling

Moderators:
Elena Goncharova, PhD (University of California, Davis)
and Jason Hong MD, PhD (UCLA)

7:00-8:00 A.M. - BREAKFAST

8:05 a.m. **Aging and Genetic Susceptibility in PAH: Targeting the BMP/TGF-β Pathway for Therapeutic Innovation**
Christophe Guignabert, PhD, INSERM, Université Paris-Saclay, France

8:35 a.m. **Abstract Presentation:**
Lyve1⁺ Interstitial Macrophages Undergo Hypoxia-Induced Reprogramming to Promote Vascular Remodeling and Pulmonary Hypertension
Caitlin V. Lewis, PhD, University of Colorado Anschutz Medical Center

8:50 a.m. **RNA Editing, a Driver of Pulmonary Hypertension**
Ke Yuan, PhD, Boston Children's Hospital and Harvard Medical School

9:20 a.m. **Aging Myeloid Cells as Modulators of Vascular Remodeling in Pulmonary Hypertension**
James D. West, PhD, Vanderbilt University

9:50 a.m. **Abstract Presentation:**
Vascular Smooth Muscle Cell-Derived Osteoprotegerin Drives Pulmonary Vascular Remodeling in an Experimental Model of Pulmonary Arterial Hypertension
Merve Keles, PhD, Imperial College London

10:05 A.M. - BREAK (30 MIN)

10:35 a.m. **Abstract Presentation:**
Fibroblast Growth Factor Signaling Modulates Vascular Smooth Muscle Plasticity and Protects Against Hypoxia-Induced Pulmonary Hypertension
Kel Vin Woo, MD, PhD, Washington University St Louis

10:50 a.m. **A Question of Age: Chromatin Remodeling in PAH**
Lahouaria Hadri, PhD, Icahn School of Medicine at Mount Sinai

11:20 a.m. **Abstract Presentation:**
IL-17 Signaling in Human and a Rodent Model of Chronic Thromboembolic Pulmonary Hypertension (CTEPH)
Yen-Rei Yu, PhD, University of Colorado, Anschutz Medical Campus

11:35 a.m. **Time Matters: Circadian Regulation of Vascular Aging and PH Progression**
Andrew J. Bryant, MD, University of Florida

Saturday, September 6, 2025 – Afternoon Sessions

12:05 p.m. **Abstract Presentation:**
The Loss of miR-153 Induces Endothelial-to-Mesenchymal Transition to Compromise Vascular Integrity
Aleksandra Babicheva, PhD, University of Minnesota

12:30 P.M. - LUNCH

Saturday, September 6, 2025 – Afternoon Sessions

SESSION VI: Mechanics, Biophysics and Energetics

Moderators:
Zhiyu Dai, PhD (Washington University Medical Center)
and Yen-Rei A. Yu MD, PhD (University of Colorado Anschutz Medical Campus)

3:00 p.m. **Proximal Pulmonary Artery Stiffening as a Biomarker of Cardiopulmonary Aging**
Edward P. Manning, MD, PhD, VA, Yale University

3:30 p.m. **mTOR Signaling and Smooth Muscle Cell Senescence in Pulmonary Vascular Aging**
Elena Goncharova, PhD, University of California, Davis

4:00 p.m. **Energetic Stress and Vascular Remodeling in Aging-Associated Pulmonary Hypertension**
Brian Graham, MD, University of California, San Francisco

4:30 P.M. - BREAK (15 MIN)

4:45 p.m. **How Mechanical Forces in the RV Impact Resident Endothelial cells**
Andrea Frump, PhD, Indiana University

5:15 p.m. **Creating Hydrogels for the Vascular Adventitia**
Chelsea Magin, PhD, University of Colorado

5:45 p.m. **Bioenergetic Failure and Mitochondrial Stress in Pulmonary Vascular Disease: Lessons from Early Life**
Girija G. Konduri, MD, MS, Medical College of Wisconsin

6:30 P.M. - DINNER

7:30 p.m. **Evening Poster Session**
Andrew J. Bryant, MD

Sunday, September 7, 2025 – Morning Sessions

SESSION VII: Emerging Therapeutics and Translational Science

Moderators:
Brian Graham, MD, PhD (UCSF)
and Caitlin V. Lewis, PhD (University of Colorado Anschutz Medical Campus)

7:00-8:00 A.M. - BREAKFAST

8:05 a.m. **Estrogen Signaling and Metabolic Modulation as Therapeutic Targets in Pulmonary Hypertension and Aging**
Tim Lahm, MD, National Jewish Health, University of Colorado

8:35 a.m. **Abstract Presentation:**
Targeting Notch4 Signaling in Pulmonary Arterial Hypertension
Bin Liu, PhD, Washington University in St. Louis

8:50 a.m. **DHEA as an Anti-Aging Pill in Pulmonary Arterial Hypertension: Potential Therapeutic Frontier**
Corey E. Ventetuolo, MD, Brown University

9:20 a.m. **Targeting Endosomal Dysfunction and Endothelial Senescence: RAB7 Activation as a Novel Therapeutic Strategy for Pulmonary Hypertension**
Laszlo Farkas, MD, The Ohio State University

9:50 a.m. **Abstract Presentation:**
A Single-cell atlas of the Human PAH Lung Identifies ITGA9 as a Candidate Regulator of Fibroblast Activation and Vascular Remodeling
Jason Hong, MD, PhD, University of California, Los Angeles

10:05 A.M. - BREAK (10 MIN)

10:15 a.m. **Aging, Algorithms, and Arteries: Using Technology to Predict and Track Pulmonary Hypertension**
Allan Lawrie, PhD, Imperial College London

10:45 a.m. **Closing Summary**
Sharon I.S. Rounds, MD, ATSF, Brown University
Troy Stevens, PhD, University of South Alabama

Detyrosinated α-tubulin protects against endothelial dysfunction and vascular remodeling.

Keytam S. Awad, Shuibang Wang, Gabriela Ferreyra, Christina Zhu, Jason M. Elinoff, Robert L. Danner

Background: Mutations in bone morphogenic protein receptor type 2 (BMPR2) are known to disrupt the cytoskeletal function of lung endothelial cells. Alpha-tubulin, a key component of microtubules, is involved in maintaining cytoskeletal structure, and post-translational modifications of α-tubulin appear to impact pulmonary vascular remodeling in PAH models. Tyrosination is a reversable post-translational modification of α-tubulin that involves the addition of tyrosine to α-tubulin by tubulin tyrosine ligase (TTL). In contrast, vasohibin 1 (VASH1) is a carboxypeptidase that detyrosinates α-tubulin. The balance of tyrosinated/detyrosinated α-tubulin reportedly impacts cell migration and angiogenesis. Disruption of the balance of tyrosinated/detyrosinated α-tubulin may contribute to the pathological changes observed in BMPR2-deficient pulmonary vascular cells and promote vessel remodeling in PAH.

Objectives: Determine whether: 1) disruption of α-tubulin detyrosination, through BMPR2 or VASH1 gene silencing, contributes to the cytoskeletal defects associated with BMPR2-deficieny; and 2) rebalancing α-tubulin detyrosination normalizes cellular phenotypes in BMPR2-silenced HPAECs.

Results: In BMPR2-silenced HPAECs, both VASH1 expression and detyrosinated α-tubulin levels were markedly decreased. Similar to BMPR2 knockdown, silencing VASH1 reduced detyrosinated α-tubulin levels and triggered a proliferative, pro-migratory, and anti-apoptotic HPAEC phenotype. Notably, co-silencing TTL in BMPR2-deficient HPAECs increased α-tubulin detyrosination and blocked the activation of ERK and AKT, two kinases previously linked to PAH development. Genome-wide expression profiling of BMPR2- and/or TTL-silenced HPAECs yielded 1180 DEGs (FDR <0.01 for both the main siBMPR2 and siTTL effect). Unsupervised hierarchical clustering revealed a total of 4 distinct gene clusters. In 2 of the clusters, co-silencing of BMPR2 and TTL normalized gene expression levels. Ingenuity Pathway Analysis (IPA) identified Rho GTPase cycle as the top canonical pathway in the cluster of genes downregulated following BMPR2 silencing and corrected (upregulated) by co-silencing TTL. FAK signaling was the top pathway in the cluster of genes upregulated following BMPR2 silencing and corrected (downregulated) by co-silencing TTL. Importantly, co-silencing BMPR2 and TTL suppressed guanine exchange factor H1 (GEF-H1), a protein normally inhibited/sequestered by detyrosinated α-tubulin that activates Rho GTPases when released. Furthermore, treatment with TPI-287, a microtubule-stabilizing agent under clinical investigation for cancer and neurodegenerative diseases, dose-dependently increased detyrosinated α-tubulin while suppressing cell proliferation and migration in BMPR2-deficient HPAECs.

Conclusions: The loss of α-tubulin detyrosination, resulting from VASH1 deficiency, may contribute to vascular remodeling. Increasing the levels of α-tubulin detyrosination, either by silencing TTL or through the use of small molecule inhibitors, presents a novel unexplored mechanism for preventing aberrant stress kinase signaling and correcting dysregulated cytoskeletal functions.

The loss of miR-153 induces endothelial-to-mesenchymal transition to compromise vascular integrity

Ibrahim Elmadbouh[1], Zhunran Zhong[1], Michael Thompson[2], Y.S. Prakash[2], Jason X-J Yuan[3], Aleksandra Babicheva[1,4]

1 The Hormel Institute, University of Minnesota, Austin, MN, USA; 2 Department of Anesthesiology and Perioperative Medicine, Mayo Clinic, Rochester, MN, USA;, 3 The Herbert Wertheim UF Scripps Institute for Biomedical Innovation & Technology, University of Florida-Scripps Research, Jupiter, FL, USA; 4 Lillehei Heart Institute, School of Medicine, University of Minnesota, Minneapolis, MN, USA

Rationale: Endothelial-to-mesenchymal transition (EndMT) is a process which converts endothelial cells (EC) into mesenchymal cells. EndMT has recently been implicated in the development and progression of pulmonary vascular remodeling in pulmonary hypertension (PH). However, the regulating mechanisms of EndMT in PH are still unknown. MicroRNA (miR), a small non-coding RNA, can regulate gene expression by targeting 3'-UTR of mRNA to inhibit mRNA translation and/or stability. We aimed to investigate the role of miRNA-153 in the regulation of EndMT in PH.

Methods: In silico analysis was perfomed by using standard online software (Targetscan.org) to predict miRs that can bind 3'-UTR of the EndMT transcription factors SNAI1/SNAI2. Lung EC were isolated from healthy subjects and patients with Group 1 PH. Normal human lung EC were either exposed to normoxia (21% O2) or hypoxia (3% O2) for 72h or treated with vehicle or TGF-beta1 (10ng/ml) for 7 days to induce EndMT. miR-153 level and EndMT profile were quantified by qPCR, Western blot or immunostaining. EdU and Tunnel assays were used to assess EC viability. EC permeability was determined by FITC-dextran assay. Continuous adherens junctions were quantified by CD-31 staining. Normal EC were transfected with miR-153 inhibitor or mimic and exposed to normoxia or hypoxia respectively for 72h. In another experiment normal EC were transfected with miR-153 inhibitor or mimic and exposed to vehicle or TGF-beta1 respectively for 7 days.

Results: In silico analysis revealed that miR-153 directly binds 3'-UTR of the human SNAI1 and SNAI2 genes. Downregulated miR-153 was correlated with increased SNAI1/2, EndMT and higher proliferation in EC from Group 1 PH patients compared to normal cells. Hypoxia or TGF-beta1 decreased miR-153, compromises EC barrier and induced EndMT by upregulating transcription factors (SNAI1/2), mesenchymal markers (SM-22 and vimentin), and downregulating EC markers (CD-31 and VE-cadherin). Transfection of cells with miR-153 inhibitor diminished miR-153 expression. Inhibition of miR-153 in EC under normoxic conditions significantly increased SNAI1/2, EndMT, vascular permeability, proliferation (and PCNA level), and decreased apoptosis. Number of continuous adherens junctions was lower in miR-153 inhibitor-transfected cells. Transfection of cells with miR-153 mimic resulted in overexpression of miR-153. Hypoxia- or TGF-beta1-induced EndMT were abolished in cells with miR-153 overexpression. Overexpression of miR-153 was able to attenuate hypoxia-induced EC survival and PCNA expression but was insufficient to restore continuous adherens junctions EC barrier function.

Conclusions: miR-153 downregulation plays an important role in the development of EndMT and compromised EC integrity implicated in PH pathogenesis.

AC6 Inhibition by Nitrosylation Is Due to Superoxide Reaction with Nitric Oxide in Hypoxic Pulmonary Hypertension

Dakshinamurti S; Maghsoudi S; Gupte TM; Smeir M; Bhatia V; Al-Khaffaf A; Hinton M; Chelikani P
Children's Hospital Research Institute of Manitoba; University of Manitoba, Winnipeg Canada

Rationale: Perinatal hypoxia or inflammation trigger persistent pulmonary hypertension of the newborn (PPHN), a catastrophic failure of neonatal pulmonary arterial relaxation in 1-6/1000 births, marked by sustained constriction of pulmonary arterial smooth muscle cells (PASMC). The adenylyl cyclase (AC) pathway (upon stimulation of Gαs-coupled receptors, AC converts ATP to cAMP) is crucial for pulmonary vasodilation. We reported that pulmonary arterial AC isoform AC6 is uniquely inhibited in hypoxia via nitrosylation of cysteine C1004, a critical residue in AC6's catalytic subunit C2, at the AC6-Gαs interface required for ATP catalysis. Hypoxia triggers protein nitrosylation; this prevents AC6 activation and cAMP generation, resulting in unchecked vasoconstriction. We hypothesized that nitroso species (sNO) arise in a spatiotemporally regulated manner from reaction of nitric oxide with superoxide generated under hypoxic conditions; and that nitrosylation interferes with functional apposition of AC6 C1 and C2 catalytic subunits.

Methods: Newborn (<24h age) primary porcine PASMC were cultured in normoxia (21% O2) or hypoxia (10% O2) for 72 hours. PASMC were also cultured from neonatal swine raised in hypoxia for 72h (PPHN), or age-matched normoxic controls. To determine reactants required for sNO generation, superoxide measured by dihydroethidium (DHE), NO/sNO by diaminofluorescein (DAF), quantified by FACS; total and protein-specific sNO quantified by biotin switch labelling, after superoxide augmentation (rotenone) with or without exogenous NO (sodium nitroprusside) or nitroso donor nitrosocysteine, NO scavenger PTIO, superoxide dismutase (PEG-SOD) or peroxynitrite scavenger FeTTPs. Using a HEK293T platform, we expressed Systematic Protein Affinity Strength Modulation (SPASM) biosensors consisting of AC6 C1-C2 constructs tethered by single-α-helical E4(R/K)4 linker, equipped with NanoBRET-based system (Nanoluciferase-EYFP) for detection of C1-C2 interaction. Effects of nitrosocysteine or nitrosoglutathione on orthosteric or allosteric activation of C1-C2 complementation were detected by BRET ratio, and cAMP generation (HTRF assay), to determine specific effect of nitrosylation on AC6 catalytic function.

Results: Hypoxia increases intracellular superoxide and total protein nitrosylation, more than does nitrosocysteine. AC6 and Gαs, but not Gαi, are nitrosylated in hypoxic PASMC. sNO generation in hypoxia is driven by superoxide, and reduced by SOD or FeTTPs, both of which restore AC activity. AC6 C1-C2 interaction corresponds with cAMP generation; treatment with nitroso donors diminishes C1-C2 complementation even to an allosteric stimulus.

Conclusions: Pulmonary arterial hypoxia promotes sNO formation via superoxide formation, which scavenges nitric oxide generating peroxynitrite. AC6 nitrosylation impairs interaction between its catalytic subunits even in absence of Gαs docking, curtailing cAMP generation. AC6 nitrosylation as a mechanism for pulmonary arterial relaxation failure is a novel target for drug discovery.

C3aR Attenuates Hypoxia Induced Pulmonary Vascular Remodeling and Pulmonary Hypertension

Christine Farrell[1], Janelle N Posey[2], Mariah Jordan[2], Caitlin V Lewis[3,4], Eva S Nozik[3,4], Cassidy A Delaney[2,4]

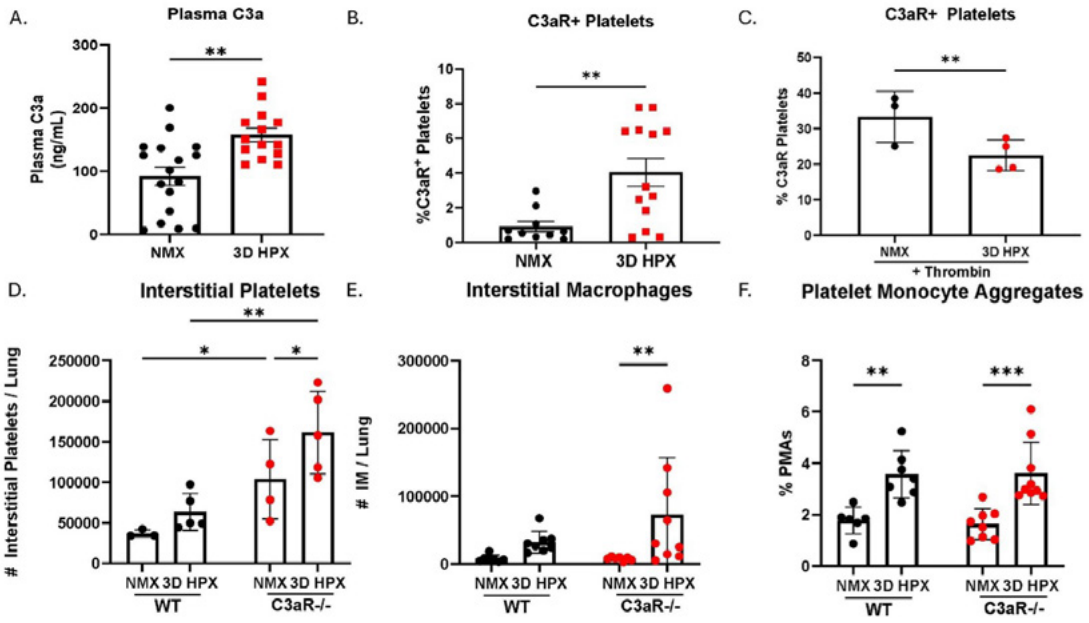
1 Division of Pulmonary Sciences and Critical Care Medicine, Department of Medicine; 2 Section of Neonatology; 3 Section of Pediatric Critical Care Medicine, Department of Pediatrics; 4 Cardiovascular Pulmonary Research Laboratories, University of Colorado Anschutz Medical Campus

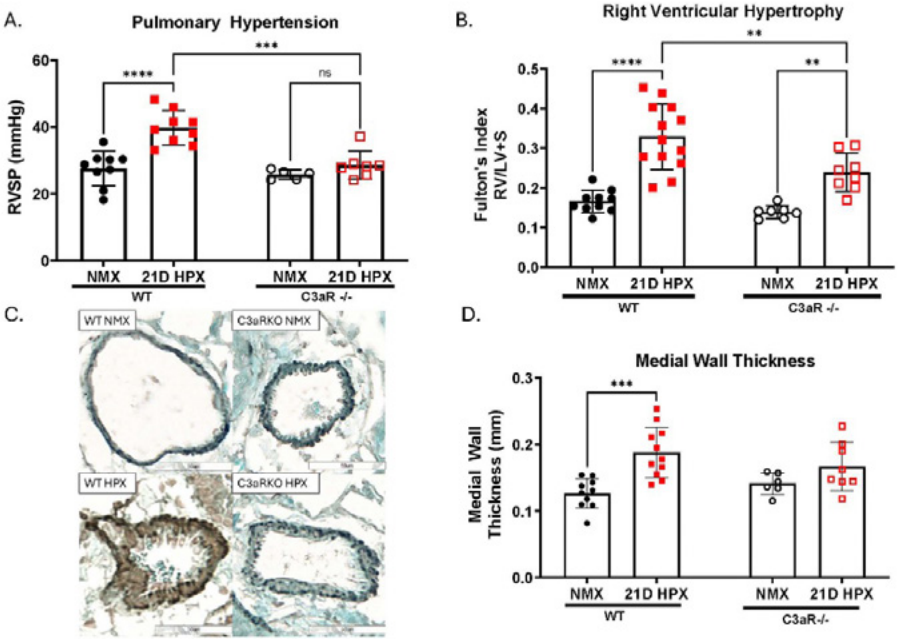
Perivascular inflammation is a consistent and essential feature of pulmonary hypertension (PH) with activation of the complement cascade emerging as a key mediator. Specifically, the complement component C3a has attracted attention as an important link between complement activation and inflammatory cell infiltration. Within the lung, the primary cell types that express the C3a receptor (C3aR) are platelets and interstitial macrophages (IMs). We hypothesize that hypoxia-induced C3aR activation leads to reduced platelet and macrophage trafficking to the lungs in response to hypoxia which mediates protection from PH.

7–12-week-old C57BL/6 wild type (WT) and global C3aR -/- mice were exposed to 10% hypobaric hypoxia for 3 or 21 days or remained in normoxia. Platelet activation and C3aR expression was assessed by flow cytometry at baseline and after stimulation with 0.1 units/mL of thrombin. RVSP was measured by closed chest RV puncture and RV hypertrophy was measured using Fulton's index (RV/LV+S). Medial wall thickness was expressed as the average width of the medial wall/average radius for α-SMA stained vessels 50 – 200μm. Lung IM, interstitial platelet, and platelet-monocyte aggregate counts were assessed by flow cytometry.

Following 3 days of hypoxia exposure, both plasma C3a (Figure 1A) and circulating platelet C3aR expression (Figure 1B) are increased. However, thrombin-induced C3aR expression is attenuated in hypoxic platelets compared to normoxic controls. C3aR -/- mice are not protected from the hypoxia-induced increase in lung interstitial platelets, lung IMs, or circulating platelet-monocyte aggregate formation. Global C3aR KO mice have attenuated hypoxia-induced PH, RV hypertrophy, and pulmonary vascular remodeling.

Our findings suggest that hypoxia exposure leads to increased plasma levels of C3a, which may engage the upregulated C3a receptor on platelets. Notably, C3aR expression is reduced on activated platelets from hypoxemic mice, indicating potential downregulation of C3aR signaling in response to sustained stimulation during hypoxia exposure. C3a plays a role in hypoxia-induced pulmonary vascular remodeling and PH, however this protection does not appear to be mediated through platelet or monocyte trafficking to the lungs. Future studies will examine whether C3aR regulates platelet and IM function within the hypoxemic lung.


A.
Plasma C3a
Plasma C3a (ng/mL)
NMX
3D HPX
B.
C3aR+ Platelets
%C3aR+ Platelets
C.
C3aR+ Platelets
% C3aR Platelets
+ Thrombin
D.
Interstitial Platelets
Interstitial Platelets / Lung
WT
C3aR-/-
E.
Interstitial Macrophages
IM / Lung
F.
Platelet Monocyte Aggregates
% PMAs


A.
Pulmonary Hypertension
RVSP (mmHg)
NMX
21D HPX
WT
C3aR -/-
B.
Right Ventricular Hypertrophy
Fulton's Index RV/LV+S
C.
WT NMX
C3aRKO NMX
WT HPX
C3aRKO HPX
D.
Medial Wall Thickness
Medial Wall Thickness (mm)

Sickle Cell Disease and Schistosomiasis Pulmonary Hypertension

Mickael [1,2,11], Jane Kabwe [3,4,11], Dara Fonseca Balladares [5,6,11], Annika Bai [5,6], Kevin Nolan [5,6], Michael Lee [5,6], Rahul Kumar [5,6], Joan Hilton [7], Joseph Phiri [3,4], Taonga Musonda [8], Katie Tuscan [2], Linda Sanders [2], Kurt Stenmark [1], Paul Buehler [9], Edford Sinkala [8], David Irwin [1,12], Sula Mazimba [10,12], Brian B. Graham [4,5,12]

1 Cardiovascular Pulmonary Laboratory, University of Colorado Anschutz Medical Campus, Aurora, CO, USA; 2 Division of Pulmonary Sciences and Critical Care Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, USA; 3 Department of Anesthesia, National Heart Hospital, Lusaka, Zambia; 4 Morningstar Clinic, Lusaka, Zambia;, 5 Division of Pulmonary and Critical Care Medicine, University of California San Francisco, San Francisco, CA, USA; 6 Lung Biology Center, Zuckerberg San Francisco General Hospital, San Francisco, CA, USA; 7 Department of Epidemiology and Biostatistics, University of California San Francisco, San Francisco, CA, USA; 8 Department of Medicine, University Teaching Hospital, Lusaka, Zambia; 9 Department of Pediatrics, University of Maryland, Baltimore, MD, USA; 10 Advanced Heart Failure and Transplant Cardiology, AdventHealth, Orlando, FL, USA; 11 Co-first authors; 12 Co-senior authors

Introduction: Pulmonary hypertension (PH) can arise from both schistosomiasis and sickle cell disease (SCD). Schistosomiasis affects >200M globally, with 90% in sub-Saharan Africa. SCD has a prevalence of >7M, with 79% in sub-Saharan Africa. The potential for synergistic pathology has not been determined.

Methods: Subjects with schistosomiasis hepatosplenic disease in Africa for PH are being screened for PH in a research protocol; a subject with sickle cell trait was identified. The individual subsequently underwent clinically indicated RHC. SCD (Berkeley) model mice were Schistosoma exposed using a protocol that causes PH in wildtype mice, and the phenotype determined.

Results: A 40yoF in Zambia had history of lake water exposure, sickle cell trait, esophageal variceal bleeding, and abdominal ultrasonography consistent with hepatosplenic schistosomiasis. Screening data suggested the possibility of PH (Table). She subsequently underwent RHC (Table), with mPAP=21mmHg diagnostic of PH, normal PCWP, elevated cardiac output, and normal PVR. SCD mice did not develop Schistosoma-induced PH, despite increased IL-4, indicating a preserved Type 2 immune response (Figure).

Conclusions: We identified a patient with concurrent sickle cell trait and schistosomiasis liver disease, who on RHC had PH, albeit largely due to high cardiac output. We did not find evidence of a synergistic PH phenotype between SCD and acute schistosomiasis exposure in a pre-clinical model.

Clinial assessment parameters of a subject with schistosomiasis hepatosple disease and sickle cell trait.

Clinical parameters	
6 minute walk distance	300m
NT-proBNP	2,602 pg/mL
Echocardiography parameters	
Tricuspid regurgitant jet velocity	2.65m/sec
Right ventricular systolic pressure	32 mmHg
Tricuspid annular plane systolic excursion	25.5mm
Estimated left ventricle ejection fraction	68%
Right heart catheterization parameters	
Right atrial pressure	0-1mmHg
Right ventricle pressure (systolic/diastolic)	33 / 1 mmHg
Pulmonary artery pressure (systolic/ diastolic/mean)	31 / 10 / 21 mmHg
Cardiac output (thermodilution)	9.8 L/min
Pulmonary vascular resistance	0.92 WU

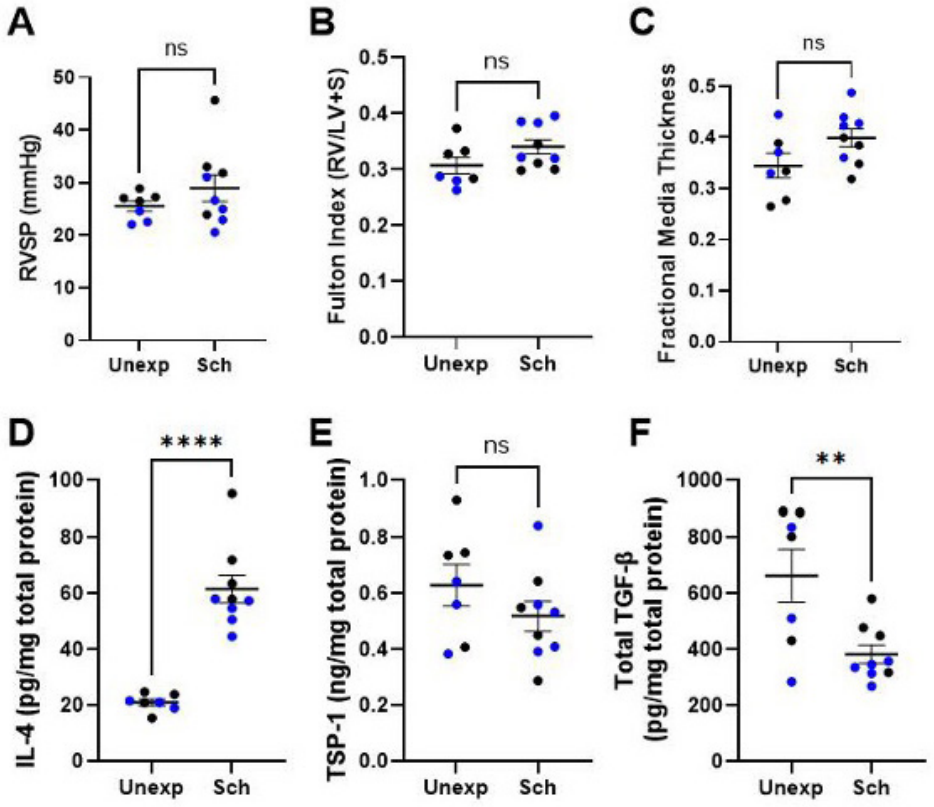


Figure. Phenotype of sickle cell disease model mice experimentally exposed to *S. mansoni*. (A) Right ventricle systolic pressure. (B) Right ventricle hypertrophy. (C) Fractional media thickness of the pulmonary vessels. (D-F) Whole lung lysate concentration by ELISA of IL-4, TSP-1 and total TGF-β. Unexp: unexposed; Sch: intraperitoneal and intravenous *S. mansoni* egg exposure. Mean +/- SEM shown; blue points are female mice and black points are male mice; t-test *P* values shown; ns: not significant; **: $P<0.01$; ****: $P<0.0001$.

A single-cell atlas of the human PAH lung identifies ITGA9 as a candidate regulator of fibroblast activation and vascular remodeling

Adam Brownstein, Brenda Wong, Tammy Graves, Micheala A Aldred, Zhiyu Dai, Xia Yang, Mansoureh Eghbali, Jason Hong.

Pulmonary arterial hypertension (PAH) is marked by progressive vascular remodeling, but key cell type–specific regulatory programs driving this process remain poorly defined. We performed single-nucleus RNAseq of 67 PAH and donor lungs to generate the largest cell atlas of the human PAH lung to date, capturing all major vascular, immune, and stromal subpopulations. Myofibroblasts were significantly expanded in PAH and enriched for epithelial-to- mesenchymal transition and PAH-associated genetic variants. Ligand–receptor

interaction and latent factor analysis identified a signaling program enriched for endothelial–stromal crosstalk and TGFβ signaling, correlating with histologic measures of remodeling. Trajectory analysis revealed that adventitial fibroblasts had the highest developmental potential among stromal cells. In a focused trajectory analysis modeling their transition to myofibroblasts, ITGA9 emerged as a top driver gene, with expression correlating with clinical severity. Spatial transcriptomics confirmed ITGA9 expression in myofibroblasts within remodeled vessels. ChIPseq demonstrated SMAD3 and SP1 binding at the ITGA9 promoter, supporting transcriptional regulation by TGFβ–related pathways.

ITGA9 was elevated in PAH across independent datasets, including bulk lung tissue, animal models, and peripheral blood. Regulatory variants linked to ITGA9 expression in myofibroblasts also correlated with disease severity. These findings from an unbiased atlas-scale single-cell analysis of the PAH lung identify ITGA9 as a transcriptionally regulated driver of fibroblast remodeling and nominate it as a potential biomarker and therapeutic target in PAH.

Targeting 14-3-3 Protein Mediated Endothelial-Pericyte Crosstalk as a Therapeutic Strategy in Pulmonary Hypertension

Mathews V. Varghese, Maki Niihori, Olga Rafikova, Ruslan Rafikov and Joel James*

Division of Pulmonary, Critical Care, Sleep, and Occupational Medicine, Department of Medicine, Indiana University, Indianapolis, IN.

*Email: *jamesjoe@iu.edu*

Background: Protein communication often occurs through thermodynamic interactions, either via direct contact or signaling intermediates. The 14-3-3 protein family represents such signaling, mediating inter-cellular communication. Despite being highly conserved across species, including plants, their biological function remains poorly understood. The lungs are made up of several cellular types and at the capillary level, endothelial cells (ECs) and pericytes maintain close interactions essential for vascular homeostasis. Inter cellular miscommunication leads to vascular diseases like pulmonary hypertension (PH), a fatal disease with no cure. We identified a novel role for 14-3-3 proteins in the pathogenesis of pulmonary hypertension, positioning them as a promising therapeutic target.

Hypothesis: We hypothesized that aberrantly expressed 14-3-3 proteins from endothelial cells bind to pericytes, triggering a pathological, proliferative phenotype. Furthermore, we tested whether disrupting 14-3-3 signaling could reverse disease progression in pulmonary hypertension.

Methods: We used NFU1 (G206C) mutant rats, a model that spontaneously develops PH and vascular remodeling. Single-cell RNA sequencing (scRNA-seq) was performed to profile lung cell populations and map cellular interactions. Seahorse was used to assess metabolic function. R18, a peptide inhibitor of 14-3-3 interactions, was used to block downstream signaling.

Results: ECs from NFU1 rats exhibited markedly increased expression and secretion of 14-3-3 proteins, consistent with observations in ECs from human PH lungs. Confocal imaging revealed significant disruption of EC-pericyte associations in the NFU1 rats, accompanied by a pericyte phenotype characterized by increased proliferation and migration. scRNA-seq analysis further demonstrated that pericytes in NFU1 lungs underwent a phenotypic transition toward a “smooth muscle like” cell state, evidenced by upregulation of canonical SMC markers such as calponin-3 and smooth muscle myosin heavy chain. CellChat interaction analysis confirmed a substantial reduction in EC- pericyte communication in the NFU1 rats.

In-vitro exposure of pericytes to 14-3-3 proteins induced mitochondrial fragmentation, impaired oxidative phosphorylation, and enhanced glycolysis, which are metabolic hallmarks of a proliferative phenotype. These pericytes also exhibited increased proliferative and invasive capacity, supporting a functional shift toward pathogenic behavior.

Therapeutic intervention using a peptide inhibitor (R18) to disrupt 14-3-3 signaling, significantly attenuated pulmonary vascular remodeling, reduced right ventricular systolic pressure, and improved pulmonary hemodynamics in NFU1 rats. scRNA-seq following R18 treatment revealed restoration of EC-pericyte interactions and reversal of the pathological pericyte phenotype.

Conclusions: Dysregulated 14-3-3 signaling drives pericyte proliferation and phenotypic transformation in pulmonary hypertension. Therapeutically targeting 14-3-3 proteins offers a novel and promising strategy to reverse vascular remodeling in this devastating disease.

Vascular Smooth Muscle Cell-Derived Osteoprotegerin Drives Pulmonary Vascular Remodelling in an Experimental Model of Pulmonary Arterial Hypertension

Merve Keles[1*], Laura E. West[2*], Nadine D. Arnold[2*], Astrid Weiss[3], Julia Baldauf[3], Nadiya V. Mykytyuk[1], Helena A. Turton[4], Josephine A. Pickworth[2], Amira Zawia[2], A.A. Roger Thompson[2], Sheila E. Francis[2], Ralph T. Schermuly[3], Allan Lawrie[1]

1 National Heart and Lung Institute, Imperial College London, London, UK; 2 Faculty of Health, University of Sheffield, Sheffield, UK; 3 University of Giessen and Marburg Lung Centre, Justus-Liebig-University Giessen, Germany; 4 Paediatric Cardiology, Department of Medicine, Stanford University, Stanford, CA, USA

** Equal contribution*

Background: Pulmonary arterial hypertension (PAH) pathogenesis is marked by sustained vasoconstriction and structural remodeling of distal pulmonary arteries.

We have previously demonstrated that upregulation of osteoprotegerin (OPG, tnfrsf11b), a TNF superfamily member associated with vascular smooth muscle cell (VSMC) aging and senescence-associated secretory phenotype (SASP), is linked to PAH pathogenesis in both animal models and tissues from PAH patients, with circulating levels being prognostic. Earlier

studies indicate that OPG knock-out mice have a reduced PAH phenotype, and therapeutic inhibition via a human monoclonal anti-OPG antibody attenuates disease in multiple rodent models. However, the key cellular source of OPG driving PAH pathogenesis is unknown.

Methods: To investigate the cell-specific role of OPG in PAH, we generated conditional Tnfrsf11b deletion by crossing OPGfl/fl mice with PGK-cretg/+ (global-knockout), LysM-cretg/+ (myeloid), Myl2-cretg/+ (cardiac myocytes), Cdh5- cretg/+ (endothelial cells, ECs), smMHC-cretg/+ (VSMCs), Col1a2-cretg/+ (fibroblasts, FBs), to generate double transgenics. Knockouts were induced by tamoxifen injection (2mg/mouse/day for 5 days), and PAH was established using Sugen5416 (20 mg/kg)/ hypoxia (SuHx) model.

Cardiopulmonary phenotyping included echocardiography, closed-chest catheterisation, and histological analysis. To explore mechanism in VSMCs, we performed peptide-based kinase activity profiling using PamStation platform, analysing 144 serine/ threonine and tyrosine kinases, separately, in HPASMCs treated with anti-OPG antibody.

Results: PGK-cretg/+/OPGfl/fl mice phenocopied the previous results obtained with global OPG knockout (OPG-/-), demonstrating reduction in right ventricular systolic pressure (RVSP) and pulmonary vascular remodelling. Myeloid- specific deletion had no effect on RVSP and provided limited benefits in pulmonary vascular remodelling, suggesting a key role for non-myeloid sources of OPG in PAH progression. Cardiomyocyte-specific deletion did not show any benefit in either cardiac or pulmonary remodelling.

Deletion of OPG in ECs, VSMCs and FBs all significantly reduced pulmonary vascular remodelling. However, only VSMC-specific deletion (smMHC-cretg/0 / OPGfl/fl) demonstrated improved haemodynamic parameters, evidenced by reduced RVSP and pulmonary vascular resistance (PVR), indicating that VSMC-derived OPG is the primary driver of PAH pathogenesis. Kinome profiling of control and IPAH-PASMCs treated with OPG +/- anti-OPG antibody revealed IPAH specific regulation of PI3K-Akt and enrichment of pathways involving autophagy, and longevity regulation linked to cellular stress resistance and growth. Anti-OPG antibody treatment reduced AKT phosphorylation in IPAH PASMCs. These results align with emerging data implicating OPG in cellular senescence and vascular aging.

Conclusions: Together, these findings highlight VSMC-derived OPG as a critical mediator of pulmonary vascular remodelling in PAH pathogenesis and suggest that targeting OPG via a therapeutic antibody is mediated through PI3K-Akt signalling.

Leptin Upregulates Bone Morphogenetic Protein Receptor-2 (BMPR2) and Suppresses Growth Differentiation Factor 15 in an Endothelial Model of Pulmonary Arterial Hypertension.

Daniels Konja,[1] Keytam S Awad,[1] Shuibang Wang,[1] Colin D Knight,[1] Gabriela A Ferreyra,[1] Kadija Hersi,[2] Jason M Elinoff,[1,2] Robert L Danner[1,2]

1 Critical Care Medicine Department, NIH Clinical Center; and 2 Critical Care Medicine and Pulmonary Branch, National Heart Lung and Blood Institute (NHLBI), National Institutes of Health, Bethesda, MD 20892 USA

Introduction: Pulmonary arterial hypertension (PAH) is a rare, fatal disease affecting the distal precapillary pulmonary arterioles. BMPR2 mutations cause 70% of heritable PAH and BMPR2 expression is significantly reduced in plexiform lesions of patients with idiopathic PAH. In addition to genetic and other causes, obesity has been investigated as a PAH risk factor. Obesity increases leptin levels, and results in oxidative stress, chronic inflammation, and endothelial dysfunction, which may exacerbate PAH. Notably, leptin can regulate growth differentiation factor-15 (GDF15), a pro- inflammatory, stress-induced cytokine/adipokine and TGFbeta ligand family member that has been associated with PAH severity. Here, leptin effects on BMPR2 and GDF15 expression was investigated in human pulmonary artery endothelial cells (PAECs) with and without BMPR2 deficiency.

Methods: Human PAECs were transfected with non-targeting siRNA or siRNA targeting BMPR2 for 48h, followed by incubation with vehicle or recombinant human leptin (10 ng/mL) for 24h. RNA and protein were harvested, and quantitative PCR and Western blotting were performed to determine the mRNA and protein expression of BMPR2, leptin receptor (LEPR), and GDF15. Secreted GDF15 was measured in cell culture supernatants by ELISA. Samples have been collected for genome-wide expression profiling to determine impact of leptin treatment in the presence and absence of BMPR2 silencing.

Results: Human PAECs were found to express a functional LEPR, but as expected, not leptin. Leptin treatment induced LEPR mRNA expression and upregulated BMPR2 mRNA and protein expression. These effects were reduced in LEPR-silenced cells. Additionally, BMPR2 silencing was partially rescued by leptin exposure, suggesting that leptin signaling might exert beneficial effects on plexogenic pulmonary arteriopathy. BMPR2 loss alone significantly induced GDF15 expression and secretion, which was suppressed by leptin treatment. In contrast, leptin induced GDF15 in control PAECs, indicating that leptin regulation of GDF15 was context dependent. Recombinant human GDF15 decreased BMPR2 and LEPR mRNA and protein expression. Silencing GDF15 had no significant effect on BMPR2 but significantly upregulated LEPR expression.

Conclusions: Leptin, acting via the LEPR, upregulates BMPR2 and GDF15 in normal human PAECs, but suppresses GDF15 in a BMPR2 loss model of PAH. The context dependent regulation of GDF15 and possibly other TGFbeta ligand family members by leptin has potential implications for how obesity may variably impact PAH prognosis and outcome.

Estrogen receptor alpha inhibits right ventricle cardiomyocyte NLRP3 inflammasome activation and restores right ventricular contractile function in low estrogen states

Fais, RS; Palotta, EDN; Kopf, KW; Massad, KM; Hoffer, C; Walts, AD; Givens, S; Bourgeois, A; Woulfe, KC; Boucherat, O; Ogle, B; Bonnet, S, Lahm, T.

National Jewish Health, University of Colorado Anschutz Medical Campus, University of Minnesota-Twin Cities, Université Laval, Rocky Mountain Regional VA Medical Center

Background: The NLRP3 inflammasome is implicated in right ventricular (RV) dysfunction, particularly in low endogenous estrogen states found in males and postmenopausal females. We hypothesized that NLRP3 inhibition through estrogen receptor-alpha (ERα) enhances RV contractility and improves RV-pulmonary artery (PA) coupling.

Methods: RVs from male pre- and postmenopausal female PH patients with RV failure (RVF) were assessed for NLRP3 activation by analyzing proteomics data and staining for NLRP3 and ASC. Male or female human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) were treated with endothelin-1 (ET-1) ± 17beta-estradiol (E2). RV cardiomyocytes (RVCMs) were isolated from male and intact or ovariectomized female rats treated with monocrotaline (MCT) or pulmonary artery banding (PAB). Male or female ERα loss-of-function mutant (ERαmut) rats were employed to study the effects of ERα. RV-PA coupling was assessed by pressure-volume loops (PV-loops) in the WT and ERαmut MCT-treated rats. NLRP3 activation in RVCMs and iPSC-CMs was assessed by NLRP3-ASC colocalization and downstream targets. RVCM contractility and cytosolic calcium (c-Ca2+) were evaluated via IONOPTIX system. $P<0.05$ was considered significant.

Results: Proteomics and immunofluorescence studies revealed that upregulation of NLRP3 activity and signaling in human RVF are more pronounced in males and postmenopausal females ($p<0.05$). In ET1-treated iPSC-CMs, NLRP3 was more activated in male than female iPSC-CMs ($p<0.05$). RVCMs from male, but not female, MCT- or PAB-rats demonstrated increased NLRP3-ASC colocalization ($p<0.05$). E2 treatment prevented MCT-induced impairment of RV-PA coupling and had beneficial effects on survival. E2 treatment of male and OVX female wildtype (WT) rat RVCMs reduced NLRP3-ASC co-localization and increased Ca2+ dependent contractility ($p<0.05$). Similarly, E2 treatment in male iPSC-CMs prevented ET1-induced NLRP3 activation. E2's inhibitory effects on NLRP3 activation, impairment of RV-PA coupling, improved survival, NLRP3-induced contractile dysfunction, and c-Ca2+ in male WT rat RVCMs were abrogated in ERαmut rat RVCMs ($p<0.05$).

Conclusions: NLRP3 activation impairs RV contractile function in a sexually dimorphic manner. The E2-ERα pathway protects against this dysfunction. E2 enhances RV function and survival in both male and postmenopausal models of RVF, suggesting its potential as a therapeutic target in low estrogen states.

Loss of estrogen receptor α causes right ventricular endothelial cell dysfunction in female rats with experimental pulmonary hypertension

Jiajun Li, Vijaya Karoor, Andrea Frump, Avram Walts, Tim Lahm

Background: Right ventricular failure (RVF) determines survival in patients with pulmonary hypertension (PH). Insufficient angiogenesis and impaired RV endothelial cell (RVEC) function are key contributors of RVF development. Female patients with PH have better RV function compared to males. 17α-estradiol (E2), the most abundant female sex hormone, exerts RV-protective effects, including stimulation of angiogenesis. However, the mechanisms underlying E2's pro-angiogenic properties are unknown.

Hypothesis: Estrogen receptor (ER)α promotes RVEC angiogenic function in vitro and RV angiogenesis in vivo.

Objectives: To identify the role of ERα in RVEC angiogenic function and RV angiogenesis. Methods: RV structure and angiogenesis endpoints were assessed in male and female wild-type (WT) and ERα loss- of-function mutant (ERαMut) rats treated with monocrotaline (MCT; 60 mg/kg) or PBS. RVECs were isolated from all groups. E2 serum levels were quantified by ELISA. RV capillary density was assessed by lectin staining. Protein expression was determined by Western blot. Cell migration was assessed using scratch assay. RVEC angiogenic gene expression was measured using Qiagen RT2 Profiler PCR array. Time courses were assessed. $p<0.05$ (ANOVA or non-parametric t-test) was considered significant.

Results: In health and disease, female ERαMut rats exhibited higher serum E2 levels compared to WT rats ($p<0.05$). Ten days and four weeks after MCT, female ERαMut MCT rats demonstrated more RV hypertrophy and less capillary density compared to female WT ($p<0.05$). At both time points, female MCT ERαMut RVs exhibited a higher fraction of PCNA+ RVECs ($p<0.05$). Pathway analysis of angiogenesis array data revealed an impairment in migration pathways in ERαMut RVECs, a finding confirmed by scratch assay. Interestingly, female ERαMut RVECs exhibited increased PCNA expression and proliferation, but also higher increased apoptosis markers (caspase 3/7 activity, cleaved-PARP levels, and bax/bcl2 ratio) compared to WT ($p<0.05$), suggesting increased apoptosis with loss of functioning ERα. Single nucleus RNA-sequencing of WT and ERαMut MCT-RV tissue is currently ongoing.

Conclusions: ERα protects female RVs against capillary rarefaction and female RVECs against loss of angiogenic capacity and potentially also apoptotic cell death. RVEC effects may underlie the observed in vivo effects. Loss of ERα may result in impaired RVEC increased apoptotic RVEC death. Targeting ERα could pave the way for sex-specific treatment strategies aimed at bolstering RV adaptation in PH.

Lyve1⁺ Interstitial Macrophages Undergo Hypoxia-Induced Reprogramming to Promote Vascular Remodeling and Pulmonary Hypertension

Caitlin Lewis*[1,2], Thi-Tina Nguyen[1,2], Janelle Posey[1,3], Timothy Porfilio[1,2], Amelia Rosin[1,2], Daniel Colon Hidalgo[1,2], Christina Sul[1,2], Cassidy Delaney[1,3], Eva Nozik[1,2]

1 Cardiovascular Pulmonary Research Laboratories, University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA; 2 Section of Pediatric Critical Care Medicine, Department of Pediatrics, University of Colorado Anschutz Medical Campus and Children's Hospital Colorado, Aurora, Colorado, USA; 3 Section of Neonatology, Department of Pediatrics, University of Colorado Anschutz Medical Campus and Children's Hospital Colorado, Aurora, Colorado, USA

Pulmonary hypertension (PH) is a progressive disease characterized by extensive pulmonary vessel remodeling and elevated mean pulmonary arterial pressure, ultimately leading to right heart failure. Perivascular accumulation of interstitial macrophages (IMs) is a hallmark of PH in both patients and preclinical models, where these cells undergo transcriptional reprogramming in response to disease stimuli such as hypoxia. Notably, deposition of the extracellular matrix (ECM) component hyaluronan (HA) is also observed in perivascular regions of remodeled vessels. The HA receptor Lyve1 is expressed on a subset of IMs, and we previously demonstrated that short-term hypoxia (~10% FiO_2 for 4 days) increases Lyve1⁺IMs in mouse lungs. We hypothesize that Lyve1+IMs undergo hypoxia-induced phenotypic reprogramming that promotes vascular remodeling and PH. To begin addressing this, we performed pilot spatial transcriptomic profiling (10x Genomics Visium V2) on mouse lungs after 4 days of hypoxia vs. normoxia. In this exploratory dataset, regions enriched for Lyve1⁺IMs (identified via pan-macrophage markers and Lyve1 expression, and confirmed by immunofluorescence) showed increased expression of collagen, ECM remodeling, and proinflammatory genes in hypoxia. This gene signature was localized to a cluster observed in perivascular lung regions. Pathway analysis within this cluster suggested upregulation of ECM organization, cell migration and adhesion, platelet activation, inflammation, and cell cycle/proliferation. To assess clinical relevance, we identified perivascular Lyve1⁺IMs (CD68⁺FOLR2⁺Lyve1⁺) in lung sections from patients with idiopathic pulmonary arterial hypertension (IPAH). To evaluate their potential contribution to PH, we generated Lyve1CreCSF1Rflox/flox mice for selective depletion of Lyve1⁺IMs. We observed >50% depletion of Lyve1⁺ IMs at baseline in these mice, with minimal effects on alveolar macrophages, monocytes, or Lyve1⁻ IMs and a significant decrease in total HA+ IMs. Following 21 days of chronic hypoxia, Lyve1CreCSF1Rflox/flox mice had attenuated right ventricular systolic pressure (RVSP) compared to floxed controls. Although right ventricular hypertrophy (RVH) was not reduced at this time point, preliminary histological analysis suggests reduced muscularization of small pulmonary vessels (<50 μm, αSMA⁺).

Together, these findings suggest that Lyve1+IMs accumulate and adopt a profibrotic, proinflammatory phenotype in response to hypoxia, promoting vascular remodeling and PH. Ongoing work will define the specific roles of HA–Lyve1 signaling and elucidate how IM–vascular interactions drive pulmonary vascular pathology.

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Targeting Notch4 Signaling in Pulmonary Arterial Hypertension

Bin Liu[1], Hanqiu Zhao[1], Ankit Tripathi[1], Qi Zheng, Xinyi Zhang[1] and Zhiyu Dai[1]

1 Division of Pulmonary Critical Care and Medicine, Department of Medicine, Washington University in St. Louis, Saint Louis, MO, USA.

Background: Pulmonary Arterial Hypertension (PAH) is a lethal disease driven by pulmonary vascular remodeling, increased resistance, and right ventricular failure, with a 15–20% 1-year mortality rate in severe cases. Current vasodilator therapies targeting endothelin, nitric oxide, prostacyclin pathway, and newly approved activin inhibitor alleviate symptoms but fail to reverse vascular pathology or cure PAH. Our data show upregulated Notch4 expression in PAH lungs, but its precise contribution and therapeutic potential require further exploration.

Objectives: We aim to define the role of Notch4 signaling in PAH and to evaluate the therapeutic potential of targeting Notch4.

Methods: We assessed the effects of Notch4 inhibition (siRNA) and activation (NICD4 overexpression) in human pulmonary vascular endothelial cells (hPVECs). In vivo, we tested the effects of Notch4 deletion in Egln1CDH5CreERT2 (iCKO) mice and anti-Notch4 antibody treatment in iCKO mice with established pulmonary hypertension. We evaluated heart function and vascular remodeling via measuring right ventricular systolic pressure (RVSP), RV/LV+S, and immunostaining, respectively. We performed bulk RNA sequencing to elucidate the underlying mechanisms of Notch4-involved PAH.

Results: We discovered that Notch4 is upregulated in rodent PAH models and IPAH lungs. NICD4 overexpression in hPVECs induced cell proliferation and upregulation of arterial genes (e.g., HEY1, HES4, CXCL12). Deletion or antibody- mediated neutralization of Notch4 in vivo significantly attenuated RVSP, RV hypertrophy, and vascular remodeling in established PAH models.

Conclusions: Our findings identify that Notch4 plays a significant role in vascular remodeling and pathogenesis of PAH. Targeted inhibition of Notch4 prevents vascular remodeling and improves heart function, providing a promising therapeutic strategy for this devastating disease.

Determining Distinct Pulmonary Artery Endothelial Cell Phenotypes in PAH and COPD-PH

Mora Massad, K.[1,2,3], Ormesher, R.[1,2], Blumhagen, R[1], Wynn, E[1], Farmer, T[1], Walts, A.1, Kim, D.1, George M.P.[1], Petrache, I.[1,2], Ventetuolo, C.E. [4], Lahm, T. [1,2,3]

1 National Jewish Health, Denver, CO; 2 University of Colorado, Anschutz Medical Campus, Aurora, CO; 3 Rocky Mountain Regional VA Medical Center, Aurora, CO; 4 Brown University, Providence, RI

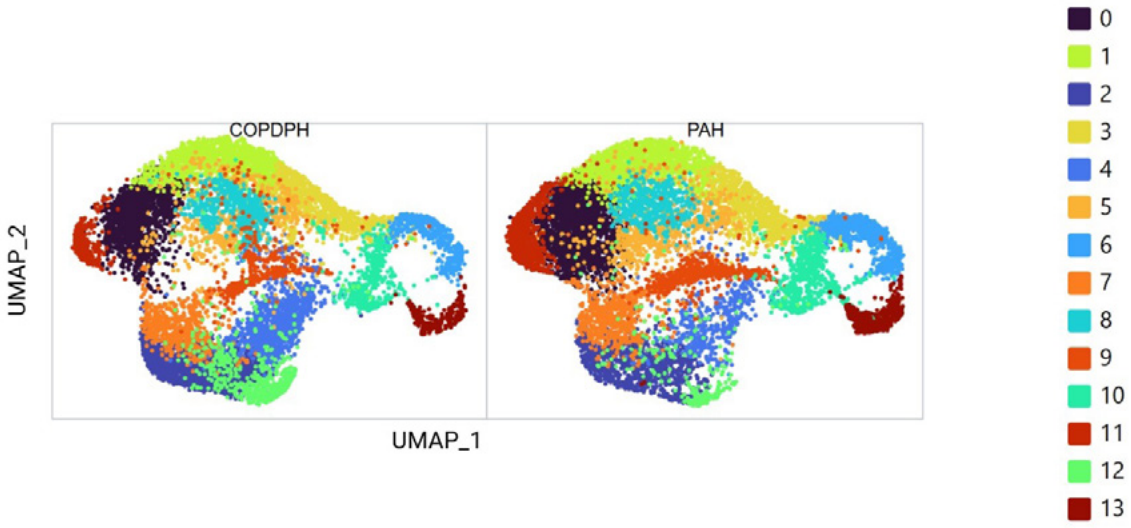
Introduction: Pulmonary artery endothelial cell (PAEC) dysfunction plays an important role in the pathogenesis of pulmonary arterial hypertension (PAH). Less is understood about their role in pulmonary hypertension due to chronic obstructive lung disease (COPD-PH). Evaluation of PAECs have previously been limited because cells are harvested from explanted lungs in end-stage disease. There is a critical need to study PAECs in earlier disease stages. We leveraged a novel method to harvest PAECs from right heart catheter (RHC) tips to study early-stage disease. PAEC phenotype was determined via single cell RNA sequencing (scRNA-seq) and functional assays. We hypothesized that PAECs are dysfunctional in COPD-PH in a pattern that is distinct from PAH PAECs by exhibiting a phenotype characterized by decreased proliferation and increased apoptotic cell death.

Methods: Participants were recruited from National Jewish Health in Denver, Colorado. Clinical data regarding PH history were obtained. PAECs were isolated from the RHC tip and sent for scRNA-seq prior to passaging. Functional assays including BrdU assay, Ki-67 assay, caspase multiplex activity ,and TUNEL assay were performed after passage 3.

Results: PAECs from 2 COPD-PH and 2 PAH patients were successfully isolated. The patients had similar disease duration (1.4 years) and severity (mean pulmonary artery pressure 40 mm Hg). Endothelial origin was confirmed via immunofluorescence. ScRNA-seq revealed that 14 distinct clusters of PAECs were present in both COPD-PH and PAH (fig. 1). Genes associated with apoptosis including FAS, BAX, BCL2 trended towards being upregulated in the COPD-PH group. Genes associated with proliferation including MYC, FOXM1, and PLK1 trended towards being more upregulated in the PAH group. Functional assays are ongoing but preliminarily suggest that PAEC apoptosis is increased in COPD- PH and PAEC proliferation is increased in PAH when compared to COPD-PH.

Conclusions: Our preliminary findings suggest that similar PAEC subtypes are present in COPD-PH and PAH but with different proportions. ScRNA-seq revealed 14 shared PAEC clusters, with trends toward increased expression of pro-apoptotic genes in COPD-PH and pro-proliferative genes in PAH. Preliminary functional data support these findings, suggesting divergent PAEC phenotypes between the two conditions that may contribute to their unique pathologies.
Funding: Borstein Family Foundation, Reuben M. Cherniack Fellowship, Colorado Pulmonary Vascular Disease Award, NIH 7R01HL144727, VA Merit Review Award 2 I01 BX00204

Funding Sources: Borstein Family Foundation, Reuben M. Cherniack Fellowship, Colorado Pulmonary Vascular Disease Award, NIH 7R01HL144727, VA Merit Review Award 2 I01 BX00204


COPDPH
PAH
UMAP_2
UMAP_1
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NOTES

CD301b⁺ cDC2s, but not Batf3⁺ cDC1s, mediate hypoxia-induced pulmonary hypertension via subset-specific activation programs

Claudia Mickael, Linda Sanders, Dara Fonseca-Balladares, Kevin Nolan, Rahul Kumar, Michael H. Lee, Kurt Stenmark, Brian B. Graham, Rubin Tuder.

Introduction: Pulmonary hypertension (PH) is a progressive vascular disease in which inflammation plays a central role. Dendritic cells (DCs) are potent antigen-presenting cells found in the lung and have been implicated in vascular remodeling, but their contribution to PH pathogenesis under hypoxia remains poorly defined. Here, we investigated how hypoxia reprograms lung classical dendritic cell subsets—cDC1s and cDC2s—and how these subsets contribute to hypoxia-induced PH.

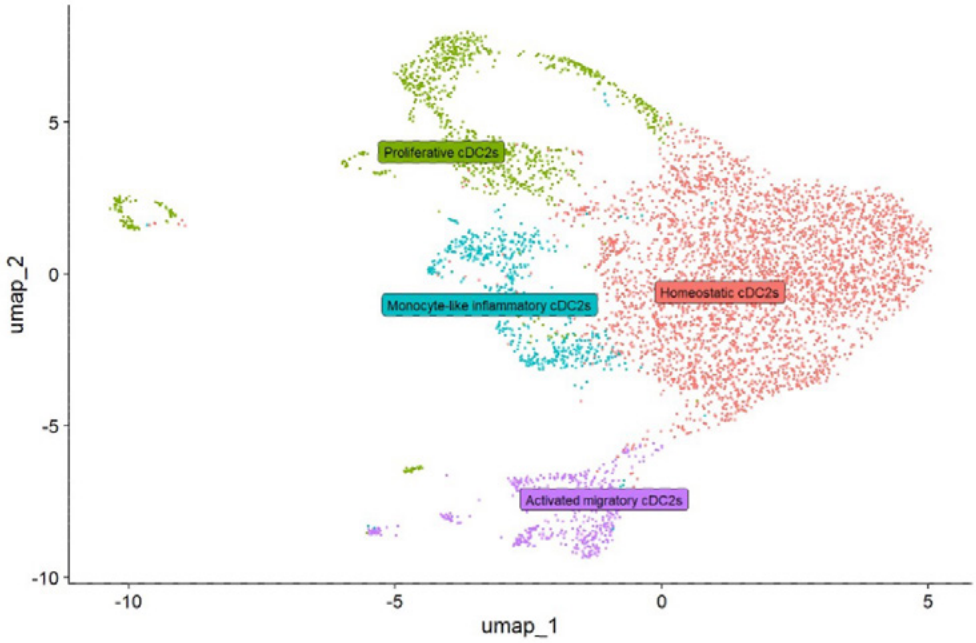
Methods: We used single-cell RNA sequencing (scRNA-seq) to profile lung DCs from 7 week old *Zbtb46*^{GFP} mice after 7 days of normoxia (NMX) or hypoxia (HYX, 10% O_2). DEGs were identified in cDC1 and cDC2 subsets, including IFN-responsive, migratory, tolerogenic, monocyte-like, proliferative, and homeostatic populations. In vivo relevance was tested using CD301b-DTR mice (to deplete CD301b⁺ cDC2s) and Batf3−/− mice (lacking cDC1s) exposed to 7 days of hypoxia. In vitro, lung cDCs were cultured under hypoxia to assess cell-intrinsic activation.

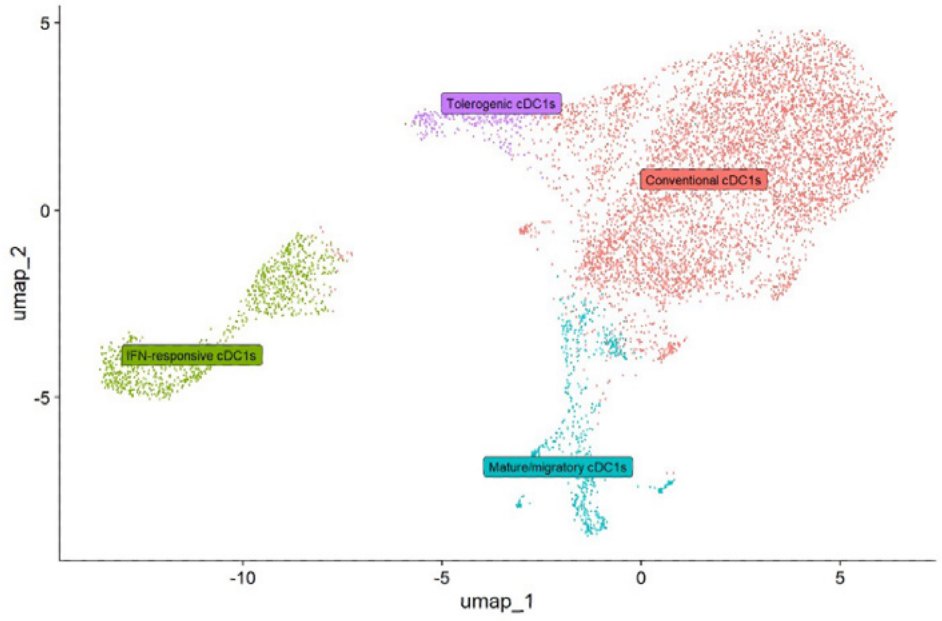
Results: scRNA-seq revealed that cDC2 subsets exhibit robust activation under hypoxia, particularly:

- Monocyte-like cDC2s upregulated Il1b, Tnf, Ccl3/4, and Ptgs2, indicating strong NF-κB–driven inflammation.
- Activated-migratory cDC2s upregulated Cd83, Cd40, Ccr7, and AP-1 components (Fos, Junb), consistent with T cell priming and lymph node migration.
- Proliferative cDC2s showed enrichment of mitotic genes (Mki67, Cdk1), suggesting hypoxia-induced expansion.
- In contrast, cDC1 subsets displayed limited pro-inflammatory responses:
- IFN-responsive cDC1s upregulated Ifit3, Irf7, and Stat1.
- Tolerogenic cDC1s expressed Il10ra, Pdcd1lg2, and Socs2.
- Overall, cDC1 activation was modest and oriented toward immune regulation.

Functionally, CD301b-DTR mice were protected from hypoxia-induced PH, implicating CD301b⁺ cDC2s as pathogenic effectors. In contrast, Batf3−/− mice developed PH similar to wild-type controls, indicating that cDC1s are dispensable in hypoxic-PH. Importantly, isolated lung cDCs exposed to hypoxia in vitro showed minimal activation, suggesting that extrinsic cues in the lung microenvironment are required for cDC reprogramming in vivo.

Conclusions: Our study demonstrates that CD301b⁺ cDC2s drive hypoxia-induced PH through subset-specific inflammatory and migratory transcriptional programs, while Batf3-dependent cDC1s are not essential. These findings position cDC2s as key immunologic effectors and potential therapeutic targets in hypoxia-associated vascular remodeling and PH.





How MEOX2 Makes Lungs New: Fibroblasts, Caps, and Air Sacs Too!

Thomas J. Taylor, Matthew R. Riccetti, Jenna Green, Kimberly Wagner, Cheng-Lun Na, Anne-Karina T. Perl

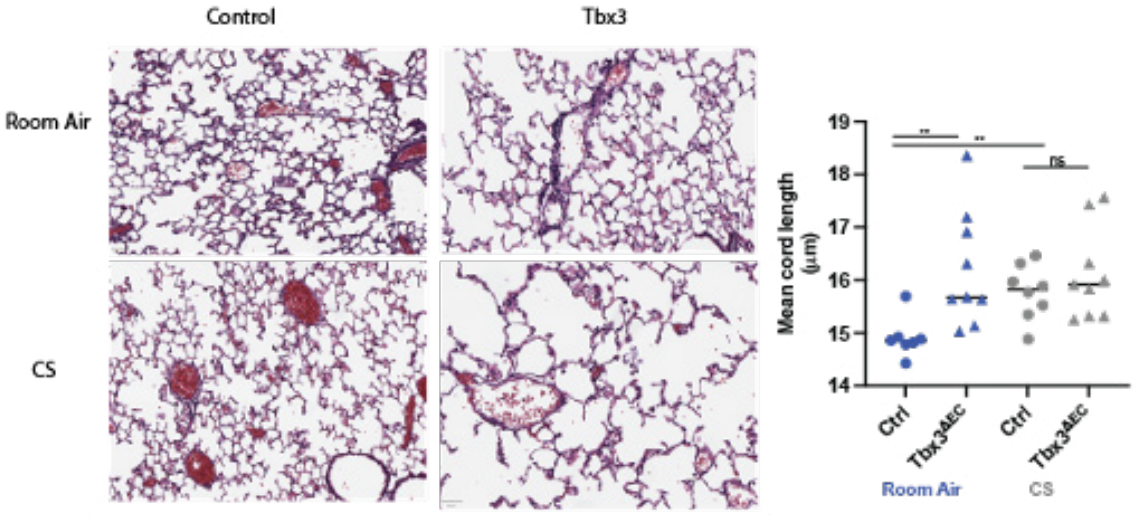
1 Division of Neonatology and Pulmonary Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA; 2 Molecular and Developmental Biology Graduate Program, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA; 3 College of Arts and Sciences, University of Cincinnati, Cincinnati, OH, USA; 4 Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH, USA

Premature infants experience a disruption of alveolarization near the end of embryonic lung development, leading to the acquisition of alveolar simplification classified as bronchopulmonary dysplasia (BPD). During alveolarization, the distal lung contains three functionally distinct populations of PDGF Receptor alpha (PDGFRA)-expressing fibroblasts: ECM- producing matrix fibroblasts, contractile secondary crest myofibroblasts (SCMF), and lipid-storing lipofibroblasts. These populations play essential roles in secondary crest formation and the maintenance of a niche for alveolar epithelial type II (AT2) self-renewal and type I (AT1) differentiation. During the second phase of alveolarization, a maturation process occurs involving septal wall thinning, SCMF loss, and microvasculature remodeling, resulting in a single-layer capillary network underneath AT1 cells. Mesenchyme homeobox 2 (MEOX2), a homeodomain transcription factor, is a signature gene of matrix Alveolar Fibroblast 1 (AF1) fibroblasts. In this study, we generated PdgfraCreER/Meox2Flx/Flx mice to delete Meox2 postnatally (Meox2Δ/Δ). Meox2Δ/Δ lungs displayed severe alveolar simplification, reminiscent of BPD, with thickened alveolar septa and capillary crowding identified via histological stains and electron microscopy. Single-cell sequencing showed a loss of AF1 signature gene expression and a shift towards an adventitial fibroblast-like phenotype, with some cells activating contractile myofibroblast gene expression. Our Meox2Δ/Δ mouse model provides insights into mechanisms regulating AF1 identity and demonstrates the matrix fibroblast to be indispensable for regulation of cell differentiation throughout the alveolar niche. It offers new perspectives on cell-cell and cell-matrix interactions upstream of myofibroblast function in secondary septation, capillary endothelial cell specification, and capillary network formation during alveolar maturation.

The role of endothelial TBX3 in the pathogenesis of emphysema.

Alexandra C. Racanelli, Samuel Chung, Amy Kuang, Tyler Lu, Raphael Lis, Augustine Choi, and Shahin Rafii.

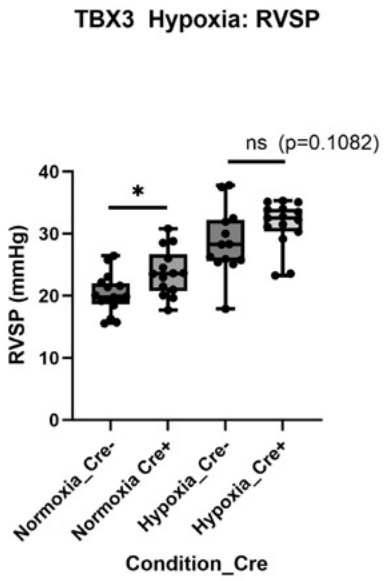
Introduction: Chronic obstructive pulmonary disease (COPD) is a heterogenous lung disease that is associated with long-term cigarette smoking and represents the fourth leading cause of death worldwide. Current therapies are limited in their ability to halt the progression of COPD and instead focus on relieving symptoms of dyspnea and airflow obstruction. Massive tissue destruction and vascular abnormalities are identified in COPD lungs as a key pathologic feature. In these studies, we explored the role of EC TBX3 in the pathogenesis of COPD/emphysema using human samples and murine models of emphysema. Methods: We performed in silico analyses of RNA sequencing datasets from the Lung Genomics Research Consortium (LGRC) in COPD and control tissue and bulk RNA sequencing of ECs obtained from COPD and control lungs samples. Additionally, we used the 9-month CS smoke model to study ECs in the disease state. We isolated lung ECs from smoke treated mice and performed RNA sequencing. We utilized a mouse line in which Tbx3 (Tbx3/iΔEC) was deleted in adult mouse ECs following injection with tamoxifen and exposed this line to our CS model. Results: TBX3 was downregulated in COPD samples and indirectly correlated with pulmonary lung function and directly correlated with severity of emphysema. The low levels of TBX3 were identified in the EC compartment in human tissue samples. Likewise, TBX3 transcriptional levels were down in ECs from mice exposed to CS. Furthermore, Tbx3/iΔEC mice developed spontaneous emphysema compared to control animals. We hypothesize that loss levels of TBX3 in PCECs drives EC dysfunction, leading to disruption of alveolar capillary matrix preventing proper repair. We are pursuing the mechanism of these disruptions in our current investigations.



The role of endothelial TBX3 in the pathogenesis of pulmonary arterial hypertension.

Alexandra C. Racanelli, Samuel Chung, Amy Kuang, Tyler Lu, Raphael Lis, and Shahin Rafii.

Introduction: Pulmonary arterial hypertension (PAH) is a rare but debilitating disease hallmarked by vascular remodeling and vasoconstriction of the pulmonary vessels resulting in progressive dyspnea, exercise intolerance, increased pulmonary vascular resistance, right ventricular failure, and premature death. Variants of PAH affect over one hundred million people worldwide and while current treatments may address symptoms or hemodynamics, most lack critical disease-modifying capabilities, calling for major advances in our understanding of PAH pathogenesis. T-box transcription factor is a lung-enriched endothelial transcription factor whose function in adult lung is largely unknown. We have preliminary data suggesting that adult mice where endothelial Tbx3 is genetically deleted develop pulmonary vascular abnormalities that lead to pulmonary hypertension. In these studies, we aimed to determine the role of a lung specific endothelial transcription factor, Tbx3, in the pathogenesis of PAH. Methods: We performed the chronic hypoxic murine model, where mice were exposed to FiO2 of 0.1 for 3-4 weeks and physiological measurments of pulmonary hypertension were assessed. We utilized a mouse line in which Tbx3 (Tbx3/iΔEC) was deleted in adult mouse ECs following injection with tamoxifen and exposed this line to our chronic hypoxia model. We isolated lung ECs from hypoxia treated mice and performed RNA sequencing. Results: Loss of endothelial TBX3 lead to spontaneous PH evidenced by elevated right ventricular systolic pressures in room air mice which was recapitulated in hypoxia mice compared to controls. We performed bulk RNA sequencing in all samples and found that loss of Tbx3 appears to drive loss of identifiy of certain ECs. In our early studies, we are finding that these ECs appear to be acap in origin. We hypothesize that loss levels of TBX3 in PCECs drives EC dysfunction, leading to disruption of alveolar capillary and development of PH. We are pursuing the mechanism of these disruptions in our current investigations.



The Two Faces of B-Catenin in the distal lung endothelium: Homeostasis, injury and regeneration

Courtney Stockman[1], Paulette Danahe Monroy Alfaro[2], Manasvi Pinnaka[3], Annika Bai[4], Anne Karina T Perl[1], Roel Nusse[5, 6, 7], Tushar Desai[2,7]

1 Neonate and Pulmonary Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA; 2 Department of Internal Medicine, Division of Pulmonary and Critical Care, and Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Stanford, CA, USA; 3 Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA, USA; 4 Department of Chemistry and Biochemistry, University of California Santa Barbara, Santa Barbara, CA, USA; 5 Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford, CA, USA; 6 Department of Developmental Biology, Stanford University School of Medicine, Stanford, CA, USA; 7 Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Stanford, CA, USA

Gas exchange in the distal lung depends on a specialized alveolar-capillary interface formed by endothelial cells (ECs) and alveolar type 1 cells, which make up ~2.3-million alveoli and function as both a gas exchange surface and a barrier to environmental insults. Endothelial integrity is critical for maintaining lung function, yet the signaling pathways that regulate EC identity and homeostasis in the adult lung remain poorly defined. Wnt-Signaling, and its effector β-Catenin, is known to regulate angiogenesis, barrier function, and cell proliferation in other vascular beds, but its role in the adult lung endothelium is not well understood. To investigate this, we used genetic mouse models to delete or stabilize β-Catenin in ECs, alongside ex-vivo lung slice cultures treated with Wnt-pathway modulators. Loss of β-Catenin led to endothelial junction remodeling, increased permeability, alveolar enlargement, and cell loss, indicating its essential role in maintaining vascular integrity.

We next asked how Wnt-signaling affects endothelial responses to injury. A subset of lung ECs displayed Wnt-activity at baseline. Stabilizing β-Catenin prior to injury enhanced EC survival and general capillary (CAP1) proliferation, but impaired differentiation into aerocytes (CAP2), suggesting a trade-off between regeneration and cell identity. Ex-vivo activation of Wnt-Signaling via Fzd4 preserved endothelial integrity, further supporting a protective role for this pathway. Together, these findings define a dual role for β-Catenin in the adult lung endothelium: maintaining adherens junctions during homeostasis and promoting endothelial survival following injury. However, prolonged Wnt-activation may compromise capillary specification. These results highlight Wnt/β-Catenin signaling as a critical regulator of lung endothelial plasticity and a potential target to enhance lung repair.

Association of RV Endocardial Radiomics with Age in Patients with Pulmonary Hypertension

Timothy C. Frommeyer, Nasteha Abdullahi, Scott Visovatti, Ping Zhang, Rebecca R. Vanderpool

Background: Right ventricular (RV) function is highly predictive of mortality in patients with pulmonary hypertension (PH). RV ejection fraction and RV end-systolic stroke volume index significantly associated with mortality but less is known about higher-order cardiac magnetic resonance (CMR) features including Radiomic features. Little is known about the change of radiomics features with age.

Hypothesis: We hypothesize that clustering on radiomic features that associate with outcomes and age will identify novel phenotypes in patients with PH.

Methods: Patients with four-chamber Cine cardiac magnetic resonance imaging were identified in the OSU CMR/RHC PH registry. Standard radiomic features were extracted from RV endocardium regions of interest (ROI) using Matlab (R2024b). Grayscale images were adjusted using histogram equalization (histeq). Shape, intensity and texture-based features were extracted across the cardiac cycle (102 features/frame). To capture time-series changes, radiomic features were assessed at end-diastole (ED), end-systole (ES), and averaged during systole (sys) and diastole (dia) (408 features per subject). Features with no variance and highly correlated features ($|r|>0.9$) were filtered out (n = 305). Clustering approaches used features identified from an XGBoost (a gradient boosting algorithm) predictive model and a Pearson correlation analysis to identify age-associated features. The relative importance of each feature was also assessed using Shapley Additive exPlanations (SHAP) values. Two clustering approaches were take 1) clustering based on XGBoost- identified features (XGBoost Clusters) and 2) a combination of XGBoost-identified and Age-associated radiomics features (Age Clusters). Kaplan Meier analysis was used to investigate associations with mortality.

Results: Radiomic features were extracted in 97 participants (age: 55±15 years) with an average RV ejection fraction of 41±14% and 25 deaths at 1 year. The radiomics XGBoost model showed good accuracy (0.89, 95%CI: 0.67-0.99). Top 3 features in the Radiomics model included a mix of shape-based, gray-level cooccurrence matrix, and first order features (Figure 1B). The radiomics features that significantly correlated with age were all first order features. As expected, decreased RVEF associates with increased mortality (Figure 2A). XGBoost clusters 2 and 3 have increased mortality compared to XGBoost cluster 1. (Figure 2B). The addition of age-associated radiomics features improved the stratification of mortality in the Age Clusters (Figure 2C). Each of the clustering approaches did group the cohort differently (Figure 2D).

Conclusions: Clustering approaches that use RV endocardial radiomics features identified novel groups that significantly associate with mortality in a small mixed cohort of patients with PH.

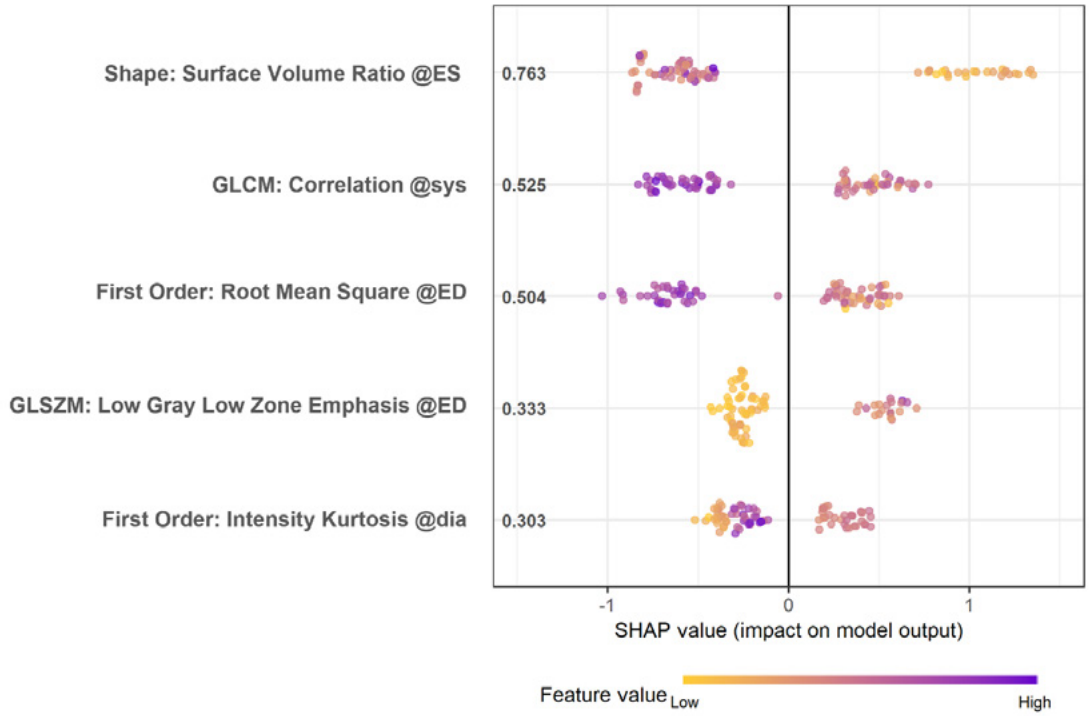


Figure 1. SHAP value magnitudes across all the samples showing the distribution of impacts of each feature in the Radiomics feature model output. The color represents the feature value (purple:high, yellow:low). GLCM: gray-level cooccurrence matrix, GLSZM: Gray level size zone matrix, @ES: values at end-systole, @sys: averaged value in the systolic phase, @ED: values at end-diastole, @dia: averaged value in the diastolic phase.

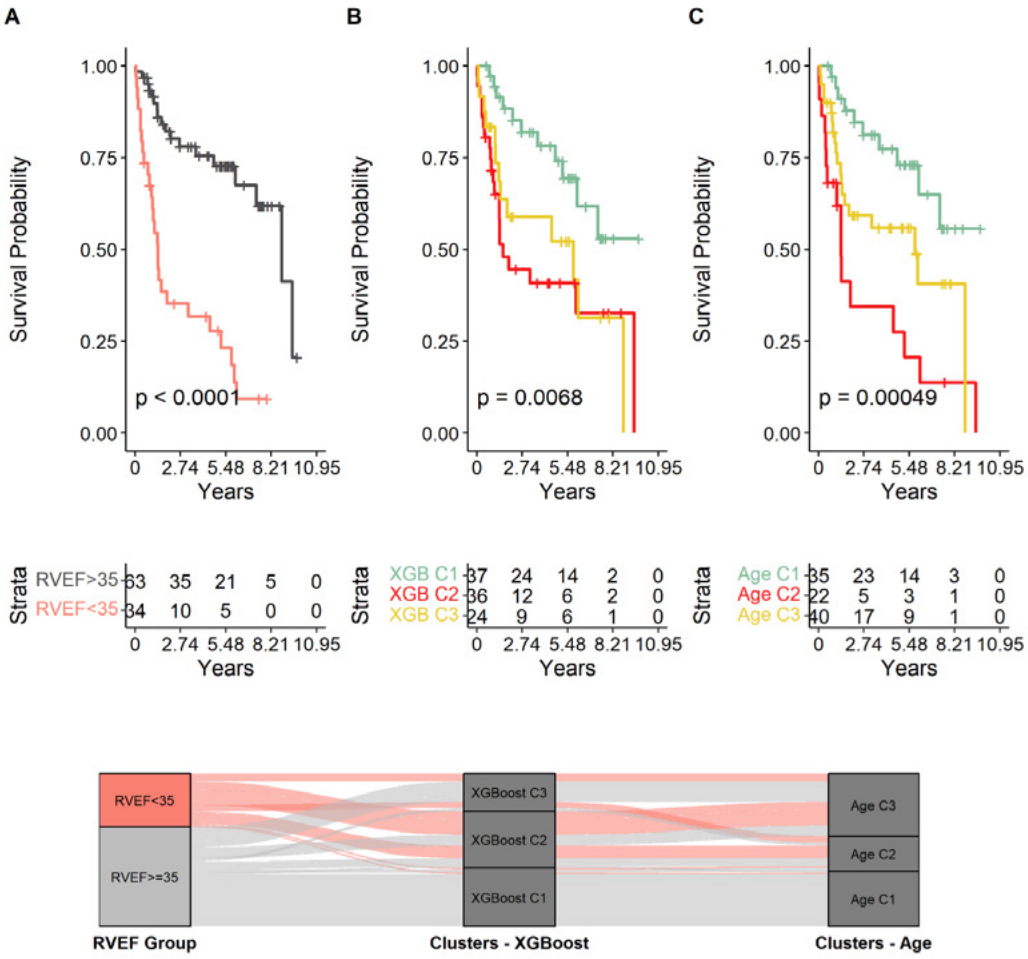


Figure 2. Kaplan Meier Survival analysis for A) RVEF, B) XGBoost clusters and C) Age Clusters. D) Alluvial plot showing the differences in the group composition between clustering approaches.

Fibroblast Growth Factor Signaling Modulates Vascular Smooth Muscle Plasticity and Protects Against Hypoxia- Induced Pulmonary Hypertension

Kel Vin Woo, MD, PhD[1,3], Jonathan Kropski, MD[2], David M. Ornitz, MD, PhD[3]

1 Division of Cardiology, Department of Pediatrics; 3 Department of Developmental Biology; Washington University School of Medicine, Saint Louis, Missouri; 2 Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee

Background: Pulmonary hypertension (PH) secondary to lung disease (Group 3 PH) is a leading cause of morbidity and mortality, particularly in hypoxia-causing conditions such as bronchopulmonary dysplasia, interstitial lung disease, chronic obstructive pulmonary disease and sleep apnea. Despite its prevalence, the molecular mechanisms driving vascular remodeling in Group 3 PH remain incompletely understood. Recent evidence implicates aberrant Fibroblast Growth Factor (FGF) signaling in the pathogenesis of PH, yet its cell-specific roles are unclear.

Methods: We utilized a physiologically relevant mouse model of Group 3 PH, exposing mice to 10% hypoxia for two weeks, followed by right heart catheterization to assess hemodynamics and histological analysis of pulmonary vasculature. Smooth muscle cell (SMC)-specific genetic manipulation of FGF receptors (FGFR1,2) and FGF2 overexpression were employed to dissect cell-type-specific functions. Complementary in vitro studies assessed hypoxia-induced proliferation and phenotypic plasticity in human pulmonary artery SMCs (HPASMCs) with FGF inhibition. Single-cell RNA sequencing (scRNA-seq) from Group 3 PH patient samples validated translational relevance.

Results: Smooth muscle FGFR1,2 inactivation exacerbated, while FGF2 overexpression conferred protection against, hypoxia- induced PH. Strikingly, SMC-specific deletion of FGFR1/2 worsened right ventricular pressures and medial thickening, indicating a critical protective role for SMC FGF signaling. FGF2 overexpression in SMCs rescued the adverse phenotype. In vitro, FGF inhibition abrogated hypoxia-induced HPASMC proliferation and promoted redifferentiation into diverse sub-phenotypes, including myofibroblast, pericyte, immune- and mesenchymal-like SMCs. scRNA-seq from patient samples confirmed expansion of these sub-phenotypes in Group 3 PH.

Conclusions: Our findings reveal a previously unrecognized dual role for FGF signaling in Group 3 PH: modulating endothelial– SMC crosstalk and directly restraining SMC plasticity and remodeling. These results identify FGF signaling as a critical regulator of pulmonary vascular homeostasis and a promising therapeutic target for Group 3 PH.

IL-17 Signaling in Human and a Rodent Model of Chronic Thromboembolic Pulmonary Hypertension (CTEPH)

Allen T; Su X, Nazo N, Roy B, Rajagopal S, Yu YR

University of Colorado, Anschutz Medical Campus, Aurora 80045, USA

Chronic thromboembolic pulmonary hypertension (CTEPH) is a deadly sequela following acute pulmonary embolism (PE). In CTEPH, the acute PE does not resolve but develops into organized "thrombi," causing pulmonary vascular remodeling, leading to progressive right heart failure and death. Cell types and signaling pathways contributing to pulmonary vascular remodeling in CTEPH are poorly understood. The lack of established and accessible small animal models has limited examination of CTEPH pathogenesis. To address this limitation, we established a rodent CTEPH model based on microsphere embolization and SU5416 treatment. After 4 weeks, we observed significant right ventricular (RV) enlargement compared to vehicle control as assessed by a substantial increase in the Fulton index. Hemodynamic measurements revealed the development of severe pulmonary hypertension (PH), characterized by elevations in pulmonary artery systolic pressure (PASP), mean pulmonary artery pressure (mPAP), and pulmonary vascular resistance (PVR), accompanied by a decrease in pulmonary artery compliance. Left ventricular end-diastolic pressure (LVEDP) was not significantly different between vehicle control vs. microsphere/SU5416-treated groups, consistent with the absence of left heart failure in the rats treated with microspheres/SU5416. On histopathology, the microsphere/SU5416 model displayed obstructive lesions in pulmonary arterioles that were absent in the vehicle- treated rats. Immunohistochemical staining revealed extensive accumulations of perivascular macrophages. Single-cell-transcriptomic analyses revealed the presence of macrophages with pro-inflammatory and pro-remodeling profiles that are enriched in IL-17 activation/signaling. This recapitulated our single-cell transcriptomic examination of human chronic thrombi, which also revealed the presence of pro-inflammatory and pro-remodeling macrophages enriched in IL-17 activation/signaling. Histological stainings of both human chronic thrombi and CTEPH rodent lung revealed elevated IL-17 protein expression in disease lesions. These findings suggest that IL-17 signaling pathways may contribute to pulmonary vascular remodeling in CTEPH by recruiting and regulating the differentiation of pro- inflammatory and pro-remodeling macrophages. Additionally, the microsphere/SU5416 rodent model serves as a valuable tool for examining aspects of CTEPH pathogenesis.

ZIP12-Mediated Zinc Signaling in PAH: Therapeutic Potential of Anti-ZIP12 Monoclonal Antibody

Lan Zhao[1], Stephanie Hopley[2], Chien-Nien Chen[1], Iyobel Kibred[1], Nicoleta Baxan[1], Richard Butt[2], Paul Hamblin[2], Martin R. Wilkins[1]

1 National Heart and Lung Institute, Faculty of Medicine, Imperial College London, London, UK; 2 Apollo Therapeutics Limited, Cambridge, UK

The zinc transporter ZIP12 is upregulated in pulmonary arterial hypertension (PAH) and contributes to vascular remodeling, metabolic dysregulation, and right ventricular (RV) dysfunction. We investigated the role of ZIP12 in PAH pathogenesis and evaluated the therapeutic potential of novel humanized monoclonal antibodies targeting ZIP12.

ZIP12 overexpression by a ZIP12-encoding plasmid induced Zn2+ influx, enhanced proliferation and metabolic reprogramming in pulmonary artery smooth muscle cells (PASMCs), associating with elevated glucose uptake, mitochondrial hyperpolarization, and increased reactive oxygen species, as well as activation of Akt pathway. ZIP12 knockdown via siRNA attenuated pulmonary hypertension and improved RV function in chronic hypoxia (Hx) and Sugen-hypoxia (SuHx) rat models. We develop a humanized anti-ZIP12 monoclonal antibody, APL-9796, that demonstrates high binding affinity and potent inhibition of zinc transport. Treatment with APL-9796 normalizes intracellular zinc levels, reduces proliferation of PAH-derived fibroblasts and PDGF-stimulated PASMCs, and reduces stress fibre formation, vasoconstriction and cytoskeletal remodeling in engineered pulmonary artery tissues and hypoxic endothelial cells. APL-9796 showed dose-dependent efficacy in attenuating pulmonary hypertension in humanized rats exposed to chronic hypoxia and significantly improved cardiopulmonary parameters in Sugen-hypoxia and monocrotaline exposed rats. Transcriptomic profiling and RT-qPCR revealed modulation of gene networks involved in proliferation, metabolism, and immune regulation, including AMPK, PPAR, and PI3K-Akt signaling pathways.

In summary, ZIP12 drives key pathogenic mechanisms in PAH through zinc-dependent signaling and mechanisms. Humanized anti-ZIP12 mAbs, demonstrate robust preclinical efficacy and support further clinical investigation in PAH patients.

PARTICIPANTS

Keytam S. Awad, PhD
keytam.awad@nih.gov
National Institutes of Health
Clinical Center, Critical Care
Medicine Department

Aleksandra Babicheva, PhD
ababiche@umn.edu
University of Minnesota

Katherine F. Beach, PharmD
kbeach@unither.com
United Therapeutics

Andrew J. Bryant, MD
Andrew.bryant@medicine.ufl.edu
University of Florida

Nunzia Caporarello, PhD
ncaporarello@luc.edu
Loyola University Chicago

Matt Casciano, PharmD
matthew.casciano@merck.com
Merck

Stephen Y. Chan, MD, PhD
chansy@pitt.edu
University of Pittsburgh School
of Medicine

Zhiyu Dai, PhD
z.dai@wustl.edu
Washington University
in St. Louis

Shyamala Dakshinamurti, MD, MS
dakshina@cc.umanitoba.ca
University of Manitoba

Jason M Elinoff, MD
jason.elinoff@nih.gov
National Heart, Lung,
and Blood Institute

Laszlo Farkas, MD
laszlo.farkas@osumc.edu
The Ohio State University

Christine Farrell, MD
jscmfarrell@gmail.com
University of Colorado

Andrea Frump, PhD
anfrump@iu.edu
Indiana University

Elena Goncharova, PhD
eagoncharova@ucdavis.edu
University of California, Davis

Kara Goss, MD
kara.goss@utsouthwestern.edu
University of Texas,
Southwestern

Brian Graham, MD
brian.graham@ucsf.edu
University of California
San Francisco

Sue Gu, MD
suegu08@gmail.com
University of Colorado

Christophe Guignabert, PhD
christophe.guignabert@inserm.fr
Université Paris-Saclay / Inserm

Lahouaria Hadri, PhD
lahouaria.hadri@mssm.edu
Ichan School of Medicine at
Mount Sinai

Akiko Hata, PhD
akiko.hata@ucsf.edu
University of California
San Francisco

Jason Hong, MD, PhD
jasonhong@mednet.ucla.edu
University of California
Los Angeles

Joel James, PhD
jamesjoe@iu.edu
Indiana University

Tatiana Kalin, MD, PhD
tatianakalin@arizona.edu
University of Arizona

Vladimir Kalinichenko, MD, PhD
vkalin@arizona.edu
University of Arizona

Harry Karmouty-Quintana, PhD
harry.karmouty@uth.tmc.edu
University of Texas Health,
Houston

Merve Keles, PhD
m.keles@imperial.ac.uk
Imperial College London

Rachel S. Knipe, MD
rknipe@mgh.harvard.edu
Massachusetts General Hospital

Girija G. Konduri, MD
gkonduri@mcw.edu
Medical College of Wisconsin

Daniels Konja, PhD
daniels.konja@nih.gov
National Institutes of Health
Clinical Center

Wolfgang W. M. Kuebler, Prof. Dr.
wolfgang.kuebler@charite.de
Charité - University Medicine
Berlin, Institute of Physiology

Maya Kumar, Phd
mayak@stanford.edu
Stanford University School
of Medicine

Grazyna Kwapiszewska, PhD
grazyna.kwapiszewska-marsh
@medunigraz.at
MedUniGraz/ILH Giessen

Tim Lahm, MD
lahmt@njhealth.org
National Jewish Health/
University of Colorado

PARTICIPANTS

Allan Lawrie, PhD
a.lawrie@imperial.ac.uk
Imperial College London

Caitlin V. Lewis, PhD
caitlin.v.lewis@cuanschutz.edu
University of Colorado

Giovanni Ligresti, PhD
ligresti@bu.edu
Boston University

Krithika Lingappan, MD, MS, PhD
kritmeds@gmail.com
University of Pennsylvania

Bin Liu, PhD
binl@wustl.edu
Washington University in St. Louis

Laura Macdougall, PharmD
lmacdougall@unither.com
United Therapeutics

Chelsea M. Magin, PhD
chelsea.magin@cuanschutz.edu
University of Colorado, Anschutz Medical Campus

Susan M. Majka, PhD
susanmajka@mac.com
National Jewish Health, Denver

Edward P. Manning, MD, PhD
edward.manning@yale.edu
VA, Yale University

Karina Massad, MD
massadk1056@gmail.com
University of Colorado/ National Jewish Health

Enid Neptune, MD, ATSF
eneptune@jhmi.edu
Johns Hopkins, School of Medicine

Terren K. Niethamer, PhD
terren.niethamer@nih.gov
National Cancer Institute

Lavannya Pandit, MD, MS
Lpandit@bcm.edu
Baylor College of Medicine / Michael E. DeBakey VA Medical Center

Anne-Karina T. Perl, PhD
anne.perl@cchmc.org
Cincinnati Children's

Markella Ponticos, PhD
m.ponticos@ucl.ac.uk
University College London

Clare C. Prohaska, MD
ccprohas@iu.edu
Indiana University

Alexandra C. Racanelli, MD, PhD
acr9004@med.cornell.edu
Albert Einstein College of Medicine

Ruslan Rafikov, PhD
rrafikov@iu.edu
Indiana University

Olga Rafikova, MD PhD
olrafi@iu.edu
Indiana University

Sharon I.S. Rounds, MD, ATSF
sharon_rounds@brown.edu
The Warren Alpert Medical School of Brown University

Kelly Schweitzer, PhD
ksschwei@iu.edu
Indiana University

Natascha Sommer, MD
natascha.sommer
@innere.med.uni-giessen.de
Justus-Liebig University Giessen

Troy Stevens, PhD
tstevens@southalabama.edu
University of South Alabama

Duncan J. Stewart, MD
djstewart@ohri.ca
Ottawa Hospital Research Institute

Courtney A. Stockman, PhD
courtney.stockman@cchmc.org
Cincinnati Childrens Hospital Medical Center

Marta T. Teixeira Gomes, PhD
magomes@iu.edu
Indiana University

Thenappan Thenappan, MD
tthenapp@umn.edu
University of Minnesota

Rebecca R. Vanderpool, PhD
rebecca.vanderpool@osumc.edu
The Ohio State University

Corey E. Ventetuolo, MD, MS, FAHA
Corey_ventetuolo@brown.edu
Alpert Medical School of Brown University

Norbert F. Voelkel, MD
nfvoelkel@gmail.com
Amsterdam Medical Centers, The Netherlands

E. Kenneth Weir, BM BCh, DM
weirx002@umn.edu
University of Minnesota

James D. West, PhD
j.west@vumc.org
Vanderbilt University Medical Center

Kel Vin Woo, MD, PhD
woo_k@wustl.edu
Washington University in St Louis

PARTICIPANTS

Yen-Rei A Yu, MD, PhD
yen-rei.yu@cuanschutz.edu
University of Colorado, Anschutz Campus

Youyang Zhao, PhD
youyang.zhao@northwestern.edu
Northwestern University-Lurie Children's Hospital of Chicago

Yang Zhou, PhD
yang_zhou@brown.edu
Brown University

Ke Yuan, PhD
ke.yuan@childrens.harvard.edu
Boston Children's Hospital

Lan Zhao, MD, PhD
l.zhao@imperial.ac.uk
Imperial College London

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