The Actelion ENTELLIGENCE Young Investigator Program is supported through an educational grant from Actelion Pharmaceuticals US, Inc.
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Dear Colleagues,

We are delighted to announce that in 2014, the Actelion ENTELLIGENCE™ Young Investigator Program chose four new young investigators to receive ENTELLIGENCE awards based on their outstanding pulmonary vascular disease-related research proposals. These awards provide support to individual young investigators at universities and research institutes in the US and Canada to conduct basic science, translational, and/or clinical research through a 12-month mentored grant. Since 2005, members of the independent Steering Committee have selected cutting-edge projects that are scientifically relevant, original, and applicable to the problem of pulmonary vascular disease. More than 40 promising researchers in the field of pulmonary vascular disease have been awarded to date.

Funded studies have targeted pulmonary vascular disease in the areas of pathophysiology, pharmacology, treatment, genetics, diagnosis, and epidemiology. Results from many of these projects have been presented at numerous key scientific meetings such as the American Thoracic Society, the American Heart Association, and the European Respiratory Society, and have been published in more than 20 peer-reviewed journals, including American Journal of Physiology, American Journal of Respiratory and Critical Care Medicine, Chest, Circulation, and Pulmonary Circulation. ENTELLIGENCE awardees have also advanced their careers in pulmonary vascular disease, with many becoming Assistant and Associate Professors of Medicine, Directors, Section Leaders, and mentors for up-and-coming young investigators.

Continuing its commitment to advancing the understanding of pulmonary vascular disease and promoting the career development of young investigators planning an academic career in pulmonary vascular disease research, the Young Investigator Program will soon begin another cycle of competition, with relevant dates shown below and on the ENTELLIGENCE website: http://entelligencemd.org (where you’ll also find an excellent new video about the program).

On behalf of the ENTELLIGENCE Steering Committee, I would like to express our gratitude to Actelion for their generous gifts to the pulmonary vascular disease research community and their ongoing commitment to basic science and clinical research in this arena.

Best regards,

Ronald J. Oudiz, MD
The Actelion ENTELLIGENCE Young Investigator Program
Supporting young investigators

The Actelion ENTELLIGENCE Young Investigator Program, established in 2005, provides opportunities for promising young investigators to promote quality medical care and enhance patients’ lives by supporting research (basic science, clinical, or translational) in the area of pulmonary hypertension (PH), specifically related to expanding knowledge of pulmonary vascular pathobiology pathways. The ENTELLIGENCE program is led by a Steering Committee comprised of leaders in the field of PH who award 12-month mentored grants of up to $100,000 to conduct basic science and clinical research. Grants are based on scientific merit, originality, feasibility, and applicability to the diagnosis and treatment of PH, conditional upon supervision by an appropriate mentor, and conducted at a university or research institute in the US or Canada. The program is consistent with Actelion’s commitment to basic science and clinical research in the area of PH.

The ENTELLIGENCE program is funded by an independent grant from Actelion Pharmaceuticals US, Inc. All decisions to fund protocols are solely decided by the ENTELLIGENCE Steering Committee and the receipt of a grant in no way requires the recipient, nor implies that the recipient is obligated to, recommend or prescribe any Actelion product.

How to submit

Applicants are invited to submit original basic or clinical investigations specifically targeting pulmonary vascular disease in the following areas: Pathophysiology, Pharmacology, Treatment, Genetics, Diagnosis, and Epidemiology. Applications are submitted electronically as a Letter of Intent. Submitted applications are reviewed by the Steering Committee and selected applicants are invited to submit full proposals. The timelines, submission procedure, and submission forms are available on the ENTELLIGENCE website (www.entelligencemd.org).

Review cycles completed: 9
Awards distributed: 46
Overview of ENTELLIGENCE Awards

Awarded 2014

**Evan Brittain, MD**
Vanderbilt University Medical Center  
Co-Investigator: Thomas J. Wang, MD  
Mentor: Anna R. Hemnes, MD  
Project Title: Dysregulation of Lipid Metabolism and Right Ventricular Function in Pulmonary Arterial Hypertension

**Joshua M. Diamond, MD**
University of Pennsylvania  
Co-Investigator: Harold I. Palevsky, MD  
Mentor: Steven M. Kawut, MD, MS  
Project Title: Clinical and Biomarker Risk Evaluation of Pulmonary Hypertension in Lung Transplantation

**R. Blair Dodson, PhD**
University of Colorado Denver Anschutz Medical Center  
Mentor: Steven H. Abman, MD  
Project Title: Intrauterine Hemodynamic Stress Mechanisms of Fetal Pulmonary Vascular Injury

**Clyde J. Wright, MD**
University of Colorado School of Medicine  
Mentor: Kurt R. Stenmark, MD  
Project Title: Role of Macrophage ET-1 Expression in the Pathogenesis of Persistent Pulmonary Hypertension of the Newborn (PPHN) Caused by Perinatal Inflammation
Overview of ENTELLIGENCE Awards

Awarded 2013

Harry Karmouty-Quintana, PhD
The University of Texas Health Science Center at Houston
Mentor: Michael R. Blackburn, PhD
Project Title: The Role of Hyaluronan in Pulmonary Hypertension Associated with Idiopathic Pulmonary Fibrosis (IPF)
Presented at 2014 Keystone Symposium on Molecular and Cellular Biology

Michael L. O’Byrne, MD
Children’s Hospital of Philadelphia
Co-Investigators: Brian D. Hanna, MD, PhD; Steven M. Kawut, MD, MS; and Russell T. Shinohara, PhD
Mentor: Jonathan J. Rome, MD
Project Title: Adverse Outcomes Associated with Cardiac Catheterization in Children with Pulmonary Arterial Hypertension

Tien Peng, MD
Hospital of the University of Pennsylvania
Mentor: Edward Morrisey, PhD
Project Title: The Role of Sonic Hedgehog (Shh) Signaling in Pulmonary Arterial Hypertension

Keivan Zandinejad, MD
Case Western Reserve University
Mentor: Jonathan S. Stamler, MD
Project Title: S-Nitrosylation Therapy to Treat Hypoxia-Induced Pulmonary Arterial Hypertension
Overview of Entelligence Awards

2013 Award Winners

From left: Michael L. O’Byrne, MD; Tien Peng, MD; Ronald J. Oudiz, MD [Program Chair]; Keivan Zandinejad, MD; Harry Karmouty-Quintana, PhD
Overview of ENTelligence Awards

Awarded 2012

Eileen Bauer, PhD
University of Pittsburgh School of Medicine
Co-Investigator: Stephen Tomlinson, PhD
Mentors: Philip M. Bauer, PhD and Timothy R. Billiar, MD
Project Title: Complement Activation as a Novel Mechanism of Endothelial Activation in PH

Joshua P. Fessel, MD, PhD
Vanderbilt University Medical Center
Mentor: James D. West, PhD
Project Title: The Role of Sirtuins and Lysine Acetylation in Pulmonary Arterial Hypertension
Presented at 2013 American Thoracic Society Conference

Kenny Schlosser, PhD
Ottawa Hospital Research Institute
Mentor: Duncan J. Stewart, MD
Project Title: Role of Extracellular Circulating MicroRNAs in Idiopathic Pulmonary Arterial Hypertension
Presented at 2013 American Thoracic Society Conference and 2012 American Heart Association meeting
Published in American Journal of Respiratory and Critical Care Medicine, 2013

Kelly J. Shields, PhD
Allegheny Health Network
Co-Investigator: Joseph M. Ahearn, MD
Mentor: Raymond L. Benza, MD
Project Title: The Role of Perivascular Adipose Tissue in Pulmonary Arterial Hypertension
Accepted for presentation at 2014 American Thoracic Society Conference
Overview of ENTELLIGENCE Awards

Awarded 2011

Jana Bagarova, PhD
Massachusetts General Hospital and Harvard Medical School
Mentor: Paul Yu, MD, PhD
Project Title: BMP9-Mediated Regulation of Endothelin-1 Expression in Vascular Endothelial Cells
Presented at 2011 American Heart Association meeting

Marco Mura, MD, PhD
University of Toronto
Co-Investigator: Marc de Perrot, MD, MSc
Mentor: John Granton, MD
Project Title: Osteopontin in Idiopathic Pulmonary Arterial Hypertension, a Biomarker and Therapeutic Target
Presented at 2013 International Society for Heart & Lung Transplantation Annual Meeting and 2013 Canadian Respiratory Conference

Salah Najm, MD
University Hospitals, Case Medical Center
Mentor: Kingman Strohl, MD
Project Title: Vascular Reactivity in Response to Acute Hypoxia: Defining Features and Mechanisms
Presented at 2012 American Thoracic Society Conference

Yon K. Sung, MD
Stanford University School of Medicine
Mentor: Mark Nicolls, MD
Project Title: The Role of Antibodies in the Pathogenesis of Pulmonary Arterial Hypertension
Overview of ENTelligence Awards

Awarded 2010

**Eric Douglas Austin, MD, MSCI**
Vanderbilt University School of Medicine
Mentor: James E. Loyd, MD
*Project Title: Sex Hormone Abnormalities in Pulmonary Arterial Hypertension*
Published abstracts: American Journal of Respiratory and Critical Care Medicine, 2011 and 2012

**Angela V. Ghatnekar, PhD**
Medical University of South Carolina
Mentor: Richard M. Silver, MD
*Project Title: The Role of GATA-6 in Pulmonary Arterial Hypertension*

**Jason Gien, MD**
University of Colorado Health Sciences Center
Mentor: Steven H. Abman, MD
*Project Title: ET-1-Rho-kinase Interactions in the Pathogenesis of Neonatal Pulmonary Hypertension*
**Overview of ENTELLIGENCE Awards**

**Awarded 2010 (cont.)**

**Michael J. Passineau, PhD**  
Allegheny Health Network  
Mentor: Raymond L. Benza, MD  
*Project Title: Gene Therapy to Drive Endogenous Biosynthesis of Prostacyclin*  
*Published abstract: Molecular Therapy Supplement, 2012*  
*Presented at 2012 American Society of Gene and Cell Therapy Annual Meeting*

**Michael York, MD**  
Boston University Medical Center  
Mentor: Harrison Farber, MD  
*Project Title: dsRNA Stimulates Toll-like Receptor-3 and Increases Endothelin-1 Production by Pulmonary Artery Endothelial Cells*  
*Published in Current Opinion in Rheumatology, 2011, Expert Review in Clinical Immunology, 2011, Annals of the Rheumatic Diseases, 2010 and 2011, and Journal of Investigative Dermatology, 2010*
OVERVIEW OF ENTELLIGENCE AWARDS

AWARDED 2009

Daniel J. Kass, MD
University of Pittsburgh
Co-Investigator: Hunter C. Champion, MD, PhD
Mentor: Mark Gladwin, MD
Project Title: Targeting the MetAP2 Pathway in Pulmonary Arterial Hypertension
Presented at 2010 and 2011 American Thoracic Society Conferences
Published in PLoS One, 2012

Sean E. McLean, MD
University of North Carolina at Chapel Hill
Mentor: Cam Patterson, MD, MBA
Project Title: Smooth Muscle Cell Related Vascular Remodeling in Pulmonary Hypertension in Congenital Diaphragmatic Hernia

Alexander R. Opotowsky, MD, MPH
Boston Children’s Hospital
Mentor: Michael J. Landzberg, MD
Project Title: The Epidemiology and Determinants of Hospitalization for Pulmonary Hypertension in the United States
Presented at 2013 American College of Cardiology meeting

Michael Eric Yeager, PhD
University of Colorado School of Medicine
Mentor: D. Dunbar Ivy, MD
Project Title: Circulating Mesenchymal Precursors in Severe PAH and the Role of Endothelin-1 in their Recruitment and Differentiation into Fibrocytes
Published in European Respiratory Journal, 2012 and Chest, 2012
Overview of ENTELLIGENCE Awards

Awarded 2008

Gaurav Choudhary, MD
Alpert Medical School at Brown University
Mentor: James Klinger, MD
Project Title: Role of Endothelin-induced PKC delta Activation in Right Ventricular Hypertrophy

Hyung J. Chun, MD
Yale University School of Medicine
Mentor: Thomas Quertemous, MD
Project Title: Role of the Apelin-APJ Pathway in Endothelin-1 Signaling and Pulmonary Arterial Hypertension
Published in Nature Medicine, 2013 and Arteriosclerosis, Thrombosis, and Vascular Biology, 2011
Presented at 2009 American Heart Association meeting and 2009 American Thoracic Society Conference

Scott D. Halpern, MD, PhD
University of Pennsylvania School of Medicine
Mentor: Brian Strom, MD
Project Title: Racial Differences in Responsiveness to Endothelin Receptor Antagonists in Pulmonary Arterial Hypertension

Sayyed A. Hamidi, MD
State University of New York, Stony Brook
Mentor: Sami I. Said, MD
Project Title: A New Combination Therapy for Pulmonary Arterial Hypertension: Bosentan and VIP
Published abstracts: American Journal of Respiratory and Critical Care Medicine, 2010 and European Respiratory Journal Supplement, 2010
Published in Respiratory Research, 2011
Overview of ENTELLIGENCE Awards

Awarded 2008 (cont.)

Sanjiv Shah, MD
Northwestern University Medical Center
Mentor: John Varga, MD
Project Title: Generic Risk Factors for Connective Tissue Disease (CTD)-Associated Pulmonary Arterial Hypertension (PAH)

Venkataramana Sidhaye, MD
Johns Hopkins University
Mentor: Larissa Shimoda, PhD
Project Title: Endothelin-1 Mediated Pulmonary Smooth Muscle Migration is Mediated by AQP1
Published in American Journal of Physiology - Lung Cellular and Molecular Physiology, 2012

Ari Lev Zaiman, MD, PhD
Johns Hopkins University
Mentor: Hal Dietz, MD
Project Title: Role of Endothelin Abrogation of TGF Signaling in the Vascular Endothelium Attenuates Hypoxia Induced Pulmonary Hypertension
Presented at 2010 American Thoracic Society Conference
Overview of ENTELLIGENCE Awards

Awarded 2007

Yabing Chen, PhD
UAB School of Medicine
Mentor: Raymond Benza, MD
Project Title: PAI-1 Regulates Vascular Remodeling in Hypoxia-Induced Pulmonary Hypertension

Christopher Fiack, MD
John A. Burns School of Medicine
Mentor: Harrison Farber, MD
Project Title: Pulmonary Hypertension due to the Left Ventricular Dysfunction

Anna R. Hemnes, MD
Vanderbilt University School of Medicine
Mentor: John Newman, MD
Project Title: The Role of Endothelin-1 in Right Ventricular Response to Pressure Overload
Presented at 2008 American Thoracic Society Conference

Jeffrey C. Horowitz, MD
University of Michigan Health System
Mentor: Victor J. Thannickal, MD
Project Title: Regulation of Myofibroblast Resistance to Apoptosis by Endothelin-1
Published in American Journal of Respiratory Cell and Molecular Biology, 2009

Meredith A. Preuss, PhD
UAB School of Medicine
Mentor: David Curiel, MD
Project Title: Downstream Redox Regulation of Endothelin B Receptor in the Pulmonary Endothelium
Published in Virology, 2013 and The Open Gene Therapy Journal, 2008
Overview of ENTELLIGENCE Awards

Awarded 2007 (cont.)

Olga Rafikova, MD, PhD
Georgia Health Sciences University
Mentor: Steven P. Tofovic, MD, PhD
Project Title: Protein Nitration and Anti-remodeling Effects of Endothelin Receptor Antagonists in Pulmonary Hypertension
Published in Free Radical Biology and Medicine, 2013

Megha H. Talati, PhD
Vanderbilt University
Mentor: Barbara Meyrick, PhD
Project Title: Effect of BMPR2 Mutation in FPAH on ET-1 and ET-1 Receptors and Smad/MAPK Activation by ET-1 Receptors in Lung ECs and PASMCs in the Mouse Model of PAH
Published in the American Journal of Physiology: Lung Cellular and Molecular Physiology, 2010
Presented at 2009 American Thoracic Society meeting (travel funded by Cardiovascular Medicine Research and Education Fund)

Yerem Yeghiazarians, MD
University of California, San Francisco
Mentor: Teresa DeMarco, MD
Project Title: Effect of Endothelin Receptor Blockade on Circulating Endothelial Microparticle Levels in Patients with Pulmonary Hypertension

Awarded 2007 (cont.)
Overview of ENTELLIGENCE Awards

Awarded 2006

Joel Glasgow, PhD
UAB School of Medicine
Mentor: David Curiel, MD
Project Title: Gene Delivery for Pulmonary Hypertension

Zhigang Hong, PhD, MD
University of Chicago
Mentor: Kenneth Weir, MD
Project Title: Endothelin-Induced Increase in Pulmonary Vascular Smooth Muscle Calcium; The Role of Calcium Channels

Peter Oishi, MD
UCSF School of Medicine
Mentor: Jeffrey Fineman, MD
Project Title: Endothelin-1 Reactive Oxygen Species Interactions in Pulmonary Hypertension

Rajni Rao, MD
UCSF School of Medicine
Mentor: Yerem Yeghiazarians, MD
Project Title: Quantitative and Qualitative Properties of Endothelial Progenitor Cells in Patients with Pulmonary Hypertension
Presented at 2007 International Society of Heart and Lung Transplantation meeting
Overview of ENTELLIGENCE Awards

Awarded 2006 (cont.)

Giuseppe Valacchi, PhD
University of Siena
Mentors: Carol Cross, MD, and Gian Paolo Pessina, Professor
Project Title: Does Tocopherol Homeostasis Play a Role in Endothelin Mediated Endothelial Dysfunction?

Roham Zamanian, MD
Stanford University School of Medicine
Mentor: Ramona Doyle, MD
Project Title: The Effect of Endothelin A and B Antagonism on Insulin Resistance and Outcomes in Patients with Pulmonary Arterial Hypertension
Evan Brittain, MD
Vanderbilt University Medical Center
Nashville, TN

Dysregulation of Lipid Metabolism and Right Ventricular Function in Pulmonary Arterial Hypertension

Introduction
Right ventricular (RV) failure is the predominant cause of death in pulmonary arterial hypertension (PAH). No RV-specific therapies are available, in part because the underlying mechanisms of RV dysfunction are poorly understood. The development of RV-specific therapeutic targets would represent a major advance in the treatment of PAH.

Preliminary Data
We have found: 1) Elevated circulating free fatty acids (FAs) in PAH patients compared to age, gender, and BMI matched controls. 2) Elevated long-chain acylcarnitines in peripheral blood in PAH patients. 3) Decreased expression of enzymes involved in carnitine shuttling in pulmonary microvascular endothelial cells with BMPR2 mutation. 4) Decreased myocardial acylcarnitines associated with worse RV function and myocardial steatosis in a murine model of PAH. 5) Marked RV lipid deposition detected in vivo in patients with PAH and in explanted hearts from humans with PAH. 6) Successful trans-cardiac blood sampling and possible decreased trans-cardiac FA consumption in PAH patients compared to control.

We hypothesize that defects in FA metabolism are common in PAH and contribute to RV failure.

We propose to test this hypothesis with the following specific aims:

1. To test the hypothesis that defects in FA metabolism are common in PAH and are associated with insulin resistance, RV function, and exercise capacity. We will perform metabolomics analysis focusing on the long-chain acylcarnitine oleoylcarnitine – the most abundant acylcarnitine in our preliminary data – in 30 patients with idiopathic PAH (IPAH) and 30 matched controls.

2. To test the hypothesis that trans-cardiac metabolic profiling will demonstrate decreased uptake of FA metabolites and increased glycolysis. In this aim, we will interrogate in vivo FA metabolism by measuring trans-cardiac (pulmonary artery wedge to coronary sinus) metabolite gradients to determine the relationship between substrate metabolism and RV function. We will prospectively sample trans-cardiac FA and glucose metabolite gradients in 20 PAH patients undergoing right heart catheterization and same-day cardiac magnetic resonance imaging (MRI).
Pulmonary arterial hypertension (PAH) is a severe, progressive disorder with a median survival of 2.8 years if left untreated. While certain therapies are effective, these treatments do not cure PAH and do not appear to benefit patients with pulmonary hypertension (PH) secondary to parenchymal lung disease (WHO Group 3). Lung transplantation remains the only available therapeutic option for PAH refractory to treatment and severe WHO Group 3 PH.

Unfortunately, PH related to either PAH or parenchymal lung disease significantly increases the risk of post-lung transplant primary graft dysfunction (PGD), a form of acute lung injury that is the major cause of early post-transplant morbidity and mortality. Patients with severe PH have double the risk of PGD after transplant compared with those without PH, and those with PAH have more than three times the risk of PGD. Despite the enormous adverse impact of PH on transplant, there is poor understanding of the clinical predictors and biochemical mechanisms linking PH to PGD, preventing the institution of potential preventative or treatment strategies before or early after surgery.

Long pentraxin-3 (PTX3) is produced by macrophages and dendritic cells as a result of interleukin-1 (IL-1) and toll-like receptor (TLR) signaling pathways, and indicates activation of innate immunity. We have preliminary data showing that higher plasma PTX3 in PH may increase the risk of PGD in these patients. We have demonstrated that elevated post-transplant plasma complement levels are associated with PGD and higher post-transplant plasma PTX3 levels are associated with increased risk of PGD. We have also found that genetic variation in PTX3 is associated with PGD risk. Furthermore, a recent case-control study demonstrated higher mean plasma PTX3 concentrations in patients with PAH compared to control subjects, suggesting that PTX3 levels may serve as an effective diagnostic biomarker for PAH. The clinical and biochemical risk factors that determine which patients with PH (including PAH and PH with parenchymal lung disease) will develop PGD will be the focus of this application.
Biomechanical forces are essential for normal fetal pulmonary vascular growth and development, but increased hemodynamic stress in utero contributes to the pathogenesis of neonatal pulmonary hypertension (PPHN). Impaired endothelial function results from high pulmonary vascular resistance in PPHN, but mechanisms through which hemodynamic stress causes the PPHN remain uncertain. At birth, rapid physiologic adaptation is required for the lung to assume its essential postnatal role of gas exchange. The normal transition of the pulmonary circulation includes hemodynamic changes of immediate and progressive fall in pulmonary vascular resistance (PVR) allowing an eight-fold increase in pulmonary blood flow. Increased oxygen tension, ventilation, and shear stress are factors that contribute to the fall in PVR at birth, largely through the release of endothelium-derived vasodilators, including nitric oxide (NO) and prostacyclin, and decreased production of vasoconstrictors, such as endothelin-1 (ET-1). Failure to develop or sustain this drop in PVR at birth leads to hypoxemia and constitutes clinical syndrome of PPHN. Successful transition of the pulmonary circulation from prenatal to postnatal life requires precise and highly responsive vascular adaption to changes in hemodynamics as well as diverse growth factors and signaling. Previous models of PPHN from our laboratory have shown that chronic hemodynamic stress alters endothelial cell phenotype as characterized by abnormal vascular tone, growth, and structure, with decreased NO production and up-regulation of ET-1. In addition, fetal pulmonary artery endothelial cells (PAEC) from PPHN sheep have persistent abnormalities of growth, angiogenesis and production of vasoactive mediators. However, biomechanical mechanisms that disrupt normal vascular development and contribute to the pathogenesis of PPHN are incompletely understood.

1) Normal fetal PAECs will exhibit a phenotype similar to PPHN PAECs in vitro—increased vasoconstriction ET-1 but reduced vasodilator NO—in pathologic shear stress environment.

2) Hypoxia will augment the adverse effect of hemodynamic stress to increase production of increased vasoconstrictors but decreased vasodilators.

3) ET-1 blockade through SiRNA or bosentan will preserve fetal PAEC phenotype in hemodynamic stress and hypoxia.
2014 Abstracts

Clyde J. Wright, MD
University of Colorado School of Medicine
Aurora, CO

Role of Macrophage ET-1 Expression in the Pathogenesis of Persistent Pulmonary Hypertension of the Newborn (PPHN) Caused by Perinatal Inflammation

PPHN affects 2.6% of births and is the most common cause of cardiopulmonary failure in the neonate. Of these patients, >40% fail medical therapy and need ECMO or die. Although exposure to inflammation (chorioamnionitis, sepsis) causes 30% of the cases of PPHN, the specific cells and signaling pathways mediating this response are poorly understood. The significance of this proposal is that we will identify novel therapeutic targets for these critically ill neonates by defining the mechanistic link between perinatal inflammatory stress and PPHN.

Whether inflammatory stress-induced ET-1 expression contributes to PPHN, and whether the use of ET receptor antagonists is indicated in these patients remains largely unexplored. Although the pulmonary endothelium has been considered the primary source of ET-1, macrophages secrete ET-1 in response to pro-inflammatory stimuli. With inflammatory stress, there is a massive influx of macrophages into the fetal lung. Additionally, the rate-limiting step of ET-1 bioavailability is gene transcription. Determining the transcriptional regulation of inflammatory-stress induced ET-1 expression may identify additional therapeutic targets. The dimeric transcription factor NFκB regulates the cellular response to inflammatory stress, and the ET-1 promoter is known to have NFκB binding sites. We hypothesize that antenatal inflammation causes PPHN in part due to NFκB regulated ET-1 expression from fetal lung macrophages.

Specific Aims:

1. Demonstrate that TLR4-NFκB signaling drives LPS-induced macrophage ET-1 expression.
   Lipopolysaccharide (LPS)-stimulated macrophages will be tested for NFκB activation and ET-1 expression. The effect of attenuated TLR4/NFκB signaling on LPS-induced ET-1 expression will be tested.

2. Establish that antenatal inflammation inducens NFκB-regulated ET-1 expression in fetal lung macrophages.
   The effect of antenatal inflammation (intraamniotic LPS) on pulmonary macrophage NFκB activation, ET-1 expression and PPHN will be determined. Mice with macrophage-specific disrupted TLR4/NFκB signaling will be assessed.

3. Test whether pharmacologic NFκB or ET receptor blockade will attenuate antenatal inflammation-induced PPHN.
   The effect of postnatal ETA or nonselective ET receptor blockade on PPHN will be compared to global NFκB inhibition in newborn mice exposed to IA LPS.
Pulmonary hypertension (PH) is a disorder affecting the vasculature of the lung that is often associated with idiopathic pulmonary fibrosis (IPF). PH is characterized by increased vascular tone and remodeling of the vasculature, including increased vascular smooth muscle mass and neo-muscularization of vessels. If left untreated, patients die as a result of right ventricular hypertrophy leading to right-sided heart failure. Increased levels of hyaluronan, a component of the lung extracellular matrix, have been observed in patients with pulmonary arterial hypertension. Hyaluronan is produced by hyaluronan synthases (HAS) and can be broken down by hyaluronidases into fragments that promote inflammation, remodeling, and angiogenesis through its interaction with hyaluronan binding proteins. However, the involvement of hyaluronan signaling in PH in IPF is not fully understood. Our preliminary data demonstrate a strong correlation between HAS2 expression and mean pulmonary arterial pressure (mPAP) in patients with IPF with and without PH. In addition, increased presence of hyaluronan is observed in remodeled vessels of patients with IPF and PH. Based on these observations our hypothesis is that: Increased hyaluronan deposition in the lungs promotes vascular remodeling in PH associated with IPF. In order to test this hypothesis, we will perform critical proof-of-concept experiments on a unique set of lung tissue derived from lung explants from patients diagnosed with IPF where right-heart catheterization was performed and a diagnosis of PH is available. Human pulmonary artery smooth muscle cells will be used to determine the effect of hyaluronan fragments on cell proliferation and migration. Finally, an experimental model of lung fibrosis and pulmonary hypertension will be utilized to generate pre-clinical data supporting the role of HAS inhibition as a potential therapy to prevent the development of PH in patients with IPF. This proposal will provide key mechanistic and pre-clinical data aimed at enhancing our understanding of how the extracellular matrix is able to participate in vessel remodeling, with the view of developing novel therapies against PH secondary to lung fibrosis.
Adverse Outcomes Associated with Cardiac Catheterization in Children with Pulmonary Arterial Hypertension

Pulmonary hypertension is a rare, but extremely morbid condition in children. Hemodynamic measurement obtained via right heart catheterization is an important tool in the diagnosis, classification, and longitudinal care of these patients. However, it appears to be a significant source of iatrogenic mortality. The risk of death in children with pulmonary hypertension appears higher than in children with other forms of heart disease and adults with pulmonary hypertension. The predictors of periprocedural morbidity and mortality are not well defined. We propose to determine the risk factors for right heart catheterization-associated adverse outcomes in a multi-center cohort study and develop a prediction rule based on these factors. We hypothesize that higher catheterization laboratory volume will be associated with lower risk of mortality, and that individual/case level factors (older age, smaller size, general anesthesia, patient status, and etiology of pulmonary hypertension) will increase the risk of adverse outcomes. Our study will utilize administrative data from 40 centers in the United States that contribute data to the Pediatric Health Information System (PHIS) database. All children ages 0-18 years with the diagnosis of pulmonary hypertension who underwent heart catheterization between 2007 and 2012 at a PHIS center will be included. We will exclude patients undergoing electrophysiology studies. Our primary outcome will be mortality within 24 hours of the catheterization and initiation of mechanical circulatory support. Identification of modifiable risk factors provides an opportunity to intervene and improve safety of catheterization in children with pulmonary hypertension and to identify centers of excellence in the field.
Tien Peng, MD
Hospital of the University of Pennsylvania
Philadelphia, PA

The Role of Sonic Hedgehog (Shh) Signaling in Pulmonary Arterial Hypertension

The recapitulation of embryonic programs characterizes a variety of diseases that manifest abnormal cellular proliferation. Unraveling the biological complexity of embryonic vascular development has the potential to provide better understanding of the pathogenesis of adult vascular diseases such as pulmonary arterial hypertension (PAH). Sonic Hedgehog (Shh) is a master regulator of tissue-tissue interaction and cell fate during both heart and lung development in utero. In my preliminary studies, I demonstrated that Hedgehog signaling remains active in the adventitial layer of the adult pulmonary vasculature, and Hedgehog-activated adventitial cells proliferate to generate vascular smooth muscle in an animal model of PAH. Based on these data, I propose that Shh promotes pulmonary vascular remodeling in PAH by activating adventitial proliferation, and subsequent adventitial differentiation into vascular smooth muscle. I will address this hypothesis using both genetic and pharmacologic inhibition of Hedgehog to define Shh’s role in an animal model of PAH.
Pulmonary hypertension (PH) frequently complicates and worsens the course of patients with advanced lung diseases. And despite many years of research, the interventions for PH remain more palliative than curative.

Chronic alveolar hypoxia associated with these diseases is a major factor in the characteristic alterations in the pulmonary and systemic circulations. Although the exact molecular mechanisms responsible for initiating and propagating these processes are not well understood, PH is recognized as having impairments in nitric oxide (NO) signaling. However, current NO-based therapies (inhaled NO and sildenafil) act exclusively via cGMP pathways, even as it is now well-accepted that the vast majority of NO’s cellular activities are mediated through protein S-nitrosylation; the covalent modification of cysteine thiols to form S-nitrosothiols (SNOs).

Disruption of S-nitrosylation is an important component in a number of pathologic conditions, particularly in disease states characterized by disruptions in oxygenation. This includes PH, where we have previously documented reduced SNO/NO bioactivity; notably, levels of S-nitrosylated hemoglobin (SNO-Hb; the main regulator of oxygen delivery) were inversely correlated with disease severity. At the same time, because of the wide spectrum of activities regulated by SNOs, resolution of aberrant S-nitrosylation provides an attractive therapeutic target for disease amelioration. Indeed, acute administration of an S-nitrosylating agent to PH patients rapidly restored SNO-Hb levels, reduced pulmonary arterial pressure (PAP), and improved systemic oxygenation.

The current study builds on the earlier work to determine the long-term benefits of S-nitrosylation therapy. We hypothesize that: 1. Disruption of SNO homeostasis in the body caused by exposure to chronic hypoxia results in elevated PAP and other organ dysfunctions seen in hypoxia-induced PH; and 2. Restoration of SNO homeostasis by administration of an S-nitrosylating agent can prevent or reverse these changes. We will test these hypotheses in a rodent model of hypoxia-induced PH. Positive findings may well lead to clinical assessment of the therapeutic efficacy of S-nitrosylating agents to treat human PH patients.
**Final Report**

**Jason Giem, MD**
Assistant Professor of Pediatrics
University of Colorado Health Sciences Center
Greenwood, CO

*Mentor: Steven H. Abman, MD*

*Endothelin-1 Rho-kinase Interactions in Neonatal Pulmonary Hypertension*

Endothelial cell dysfunction, including decreased production of vasodilators, such as inhaled nitric oxide (iNO) and increased release of vasoconstrictors, such as endothelin-1 (ET-1), contributes significantly to the pathophysiology of persistent pulmonary hypertension of the newborn (PPHN). While iNO is effective in the treatment of PPHN, 40% of infants fail to respond to iNO, making it essential to study alternate pathways important in the pathogenesis of PPHN. Failure to respond to iNO is most commonly seen in the setting of vascular remodeling, impaired angiogenesis and lung hypoplasia. ET-1, when present in physiologic amounts in the fetal lung, is in part responsible for the high fetal pulmonary vascular resistance, which is necessary for the diversion of blood from the lungs during fetal life. When present in excessive amounts, however, ET-1 plays an important role in the pathogenesis of pulmonary hypertension. In adult models of experimental pulmonary hypertension induced by chronic hypoxia and monocrotaline, ET-1 contributes significantly to the development of increased PVR and hypertensive vascular remodeling. ET-1 is also important in the pathogenesis of neonatal pulmonary hypertension. In human newborns with PPHN and in infants who died from PPHN secondary to congenital diaphragmatic hernia, ET-1 levels are increased. In addition, experimental PPHN induced by ligation of the ductus arteriosus in utero ET-1 levels as well as ETA receptor activity is markedly increased, demonstrating the importance of ET-1 in the pathogenesis of severe PPHN especially in the setting of lung hypoplasia. The goal of our proposal was to address whether ET-1 alters vascular growth in the developing lung and if excessive ET-1 signaling contributes to increased smooth muscle cell growth and vascular remodeling. We also proposed studying the cell-signaling pathways downstream from ET-1 such as rho-kinase (ROCK) to add greater mechanistic depth to our studies. We utilized isolated pulmonary artery endothelial cells (PAECs) and pulmonary artery smooth muscle cell (PASMC) from a fetal sheep model of PPHN induced by partial ligation of the ductus arteriosus in utero. Our specific aims were as follows:

1. Determine the roles of ET-1 on angiogenesis during normal lung development and if these effects are mediated through ROCK.
2. Demonstrate that increased ET-1 production by PAECs from PPHN fetal sheep increases ROCK activity and impairs angiogenesis *in vitro*.
3. Determine whether ET-1 contributes to PASMC proliferation and vascular remodeling in neonatal PPHN and if these effects are mediated through ROCK.
To achieve these aims we utilized pharmacologic treatments and SiRNAs to manipulate these cell-signaling pathways, along with a novel 3 dimensional (3D) in vitro angiogenesis assay to assess the effect of these manipulations on normal and PPHN endothelial cell function in vitro. In addition, utilizing PCR and western blot, we measured differences in ET-1 expression between normal and PPHN PAECs. Our final experiments in PAECs were to explore if the effects of ET-1 on PAEC function were mediated by ROCK. To assess whether ET-1 contributes to vascular remodeling in PPHN, similar experiments were performed in PASMC, to determine the effects of ET-1 on ROCK signaling and PASMC proliferation in PASMCs.

The studies outlined in this proposal demonstrate a role for ET-1 through interactions with ROCK, in the regulation of angiogenesis in the developing lung and contributing to impaired angiogenesis in PPHN. We found that ET-1 had no effect on PAEC growth, but decreased tube formation in normal and PPHN fetal PAECs. ET-1 protein and gene expression were increased and ETB receptor protein decreased in PPHN PAECs. ET-1 inhibition with ET-1 SiRNA, ET-1 monoclonal antibody and bosentan (non-specific ET receptor blocker), but not BQ-123 (ETA-specific receptor blocker), increased tube formation in normal and PPHN PAECs. In addition, ROCK activity was increased in PPHN PAECs, and decreased with ET-1 SiRNA and bosentan treatments. In our final set of studies, we found that ROCK inhibition in the presence of ET-1 prevented the effects of ET-1 on tube formation in vitro. These findings suggest that ET-1 impairs angiogenesis of fetal PAECs through ROCK activation, and that disruption of ET-1 ROCK interactions may improve endothelial dysfunction and increase vascular growth in severe PPHN. Interestingly ET-1 mediated ROCK activation was through ETB receptor activation, suggesting that in the setting of neonatal pulmonary hypertension with impaired vascular growth, combined ETA/ETB receptor blockade may be more beneficial than selective ETA receptor blockade alone.

Our studies of isolated PASMC demonstrate that PASMCs isolated from PPHN fetal sheep demonstrate increased proliferation. Changes in ROCK and PPARγ are in part responsible for this hyperproliferative phenotype. While studies utilizing exogenous ET-1 treatment failed to demonstrate a role for ET-1 in the regulation of PASMC growth and ROCK and PPARγ signaling, ET-1 silencing in PASMC markedly decreases PASMC proliferation in both normal and PPHN PASMCs and restores changes in ROCK and PPARγ signaling to normal.

Our findings clearly implicate ET-1 as a major contributor to vascular remodeling and impaired angiogenesis in PPHN and suggest therapeutic benefit to combined ETA and B receptor blockade in the setting of PPHN complicated by impaired angiogenesis and lung hypoplasia. While the findings outlined in this proposal were derived from in vitro studies, they provide mechanistic insights into the pathogenesis of neonatal pulmonary hypertension and confirm the importance of excessive ET-1 activity to the development of impaired angiogenesis, lung hypoplasia and vascular remodeling.
Final Report

Figure 1. Decreased 3D Tube Formation by PAECs from Normal fetal Sheep after ET-1 treatment. Growth and tube formation were assessed in Normal fetal PAECs with and without ET-1 treatment. ET-1 treatment had no effect on PAEC growth. Tube formation by PPHN PAECs was significantly decreased when compared with age-matched controls. Tube length was decreased by 27% (p<0.01) and the number of branch points/HPF was decreased by 32% (p<0.01) in PPHN PAECs. ET-1 treatment of normal PAECs decreased tube formation to similar levels seen in PPHN PAECs. Tube length decreased by 15% (p<0.01) and branch points/HPF by 32% (p<0.01) in normal fetal PAECs after ET-1 treatment.

Figure 2. Growth of normal and PPHN PASMC was assessed by performing daily cell counts for 5 days and MTT assay. Compared with controls, growth of PPHN PASMC increased by 44% on day 3 (p<0.05), 49% on day 4 (p<0.01), and 51% on day 5 (p<0.01). Silencing of ET-1 signaling with SiRNA decreased growth in both normal and PPHN PASMC.
Final Report

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Boston Children’s Hospital
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Mentor: Michael J. Landzberg, MD

Hospital Admission Volume Predicts 30-day Readmission in Pulmonary Arterial Hypertension

Background

Pulmonary arterial hypertension (PAH) is associated with a high risk for adverse outcomes. Recent quality metrics have incorporated 30 day all cause readmission rates (abbreviated as “readmission”). Little, however, is known about the frequency and predictors of readmission after PAH hospitalization.

Aims

We aimed to define the epidemiology of 30-day readmission for patients admitted to a hospital with pulmonary arterial hypertension (PAH).

Methods and Results

We examined readmission after discharge for index medical hospitalizations for adults (≥18 years-old) with a principal diagnosis of PAH (ICD-9 416.0) using 2003-2006 California Office of Statewide Health Planning and Development (OSHPD) data. Hospitals were defined as high (≥20; top tertile) or low (<20) volume based on number of index PAH hospitalizations during the study period. Covariates included age, gender, year of index hospitalization, number of comorbidities, primary insurance, and an array of specific medical conditions. Three multivariable logistic regression models were developed. The first model adjusted for age, sex and potential clinical and demographic predictors of readmission. The second model included the above covariates plus length of stay in days, primary payor, do not resuscitate status, and discharge disposition. We also developed and adjusted for a propensity score for admission to a high or low volume hospital, inclusive of all potential available covariates.
There were a total of 602 index hospitalizations with a primary discharge diagnosis of PAH. Of those, 22.8% were readmitted to a hospital within 30 days. Those readmitted tended to be older and have more medical comorbidities including diabetes mellitus, coronary disease, heart failure, interstitial lung disease and hyponatremia (Table 1). Cirrhosis, HIV and congenital heart disease were not significant predictors of readmission. Those with private health insurance were less likely to be readmitted.

Discharge from hospitals with high PAH volume was associated with a lower frequency of readmission (16.2% vs. 26.4%). Hospital PAH volume remained a significant predictor despite multivariable adjustment (Table 2).

Similarly, high PAH volume hospitals had lower 30-day rates of combined readmission or death (22.8% vs. 33.4%; OR 1.8 [1.2-2.6]). This relationship remained significant after multivariable adjustment (OR 1.8, 95%CI 1.1-2.8).

**Conclusion**

Short-term readmission is common after PAH hospitalization. Patients admitted to high volume PAH hospitals have lower rates of 30-day readmission. Identification of characteristics of high volume hospital care responsible for this are needed; alternatively, these data may support a benefit of greater centralization of PAH inpatient care.

**Tables**

**Table 1:** Characteristics of patients who were readmitted within 30 days

<table>
<thead>
<tr>
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<th>YES (22.8%)</th>
<th>NO (77.2%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>137</td>
<td>465</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>56.7±1.4</td>
<td>52.6±0.7</td>
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<tr>
<td>Length of Stay, d</td>
<td>6.5±0.5</td>
<td>5.8±0.3</td>
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<tr>
<td>Male, %</td>
<td>25.9</td>
<td>25.4</td>
<td>0.32</td>
</tr>
<tr>
<td>Number of comorbidities</td>
<td>2.9±0.1</td>
<td>2.2±0.1</td>
<td>0.003</td>
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<td>Private insurance, %</td>
<td>25.6</td>
<td>38.5</td>
<td>0.009</td>
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<td>Diabetes mellitus, %</td>
<td>27.7</td>
<td>18.1</td>
<td>0.02</td>
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<td>Coronary disease, %</td>
<td>19</td>
<td>19.8</td>
<td>0.02</td>
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<td>Heart failure, %</td>
<td>51.1</td>
<td>39.4</td>
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<tr>
<td>Interstitial lung disease, %</td>
<td>10.9</td>
<td>4.5</td>
<td>0.01</td>
</tr>
<tr>
<td>Fluid disorders (hyponatremia), %</td>
<td>24.1</td>
<td>14.8</td>
<td>0.01</td>
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Table 2: 30-Day all-cause readmission by PAH hospital volume

<table>
<thead>
<tr>
<th>Volume</th>
<th>n</th>
<th>30-Day Readmission</th>
<th>Univariate OR (95% CI)</th>
<th>OR* (95% CI)</th>
<th>OR** (95% CI)</th>
<th>OR*** (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>386</td>
<td>26.40%</td>
<td>1.8 (1.2-2.8)</td>
<td>1.6 (1.04-2.6)</td>
<td>1.6 (1.02-2.5)</td>
<td>1.7 (1.1-2.8)</td>
</tr>
<tr>
<td>High</td>
<td>216</td>
<td>16.20%</td>
<td>1 (ref)</td>
<td>1 (ref)</td>
<td>1 (ref)</td>
<td>1 (ref)</td>
</tr>
</tbody>
</table>

* Adjusting for age, gender, race, year of admission, number of comorbidities, liver cirrhosis, HIV, connective tissue disease, sleep disorder, obesity, cancer, tobacco use, coronary artery disease, mitral or aortic valve disease, tricuspid valve disease, systemic hypertension, diabetes mellitus, heart failure, congenital heart disease, syncope, chronic obstructive lung disease, asthma, interstitial lung disease, sarcoidosis, deep venous thrombosis, pulmonary embolism, mechanical ventilation during hospitalization, tracheostomy, major depression, anemia, illicit drug abuse, and presence of fluid disturbances (hyponatremia).

* Adjusting for the above variables plus length of stay in days, primary payor, do not resuscitate status, and disposition (home or skilled nursing facility/rehabilitation hospital or against medical advice).

** Adjusting for a propensity score modeling the probability that a given patient would be admitted to a high or low volume hospital inclusive of all variables listed above.
Extracellular Circulating microRNAs in Pulmonary Arterial Hypertension (PAH)

Background

There is increasing interest in small non-coding RNA molecules known as microRNAs (miRNAs), as potential biomarkers, messengers and mediators in vascular disease (1,2). MiRNAs are well known for their pervasive control of gene expression within cells, but far less is known about the extracellular roles of miRNAs that circulate in the blood. Circulating miRNAs extracted from plasma represent attractive biomarker candidates because of their non-invasive source, high stability, and amenability to PCR-based amplification, which can facilitate the detection of changes at very low levels. Perturbations in the levels of specific plasma miRNAs may reflect changes in vascular homeostasis or tissue injury. Therefore, the assessment of circulating miRNAs could also provide unique insight into underlying molecular mechanisms of disease.

Aims

To identify circulating miRNAs that are concordantly altered in experimental and clinical PAH, which may serve as robust biomarkers of disease activity, and possibly contribute to the disease pathobiology. The results of these studies have been published (3), and are adapted for this report with permission of the American Thoracic Society.

Results

MiRNA screening of plasma from rats with monocrotaline (MCT)-induced PH

Quantitative PCR-based profiling arrays were used to measure the levels of 370 miRNAs in total RNA extracted from the plasma of 8 control rats, and 8 MCT-treated rats that showed significantly elevated RVSP (61 ± 5 mm Hg, MCT; 28 ± 1 mm Hg, Con; p<0.0005) and RV/(LV+S) ratio (0.31 ± 0.01, MCT; 0.22 ± 0.01, Con; p<0.0001) after 22 days. The plasma levels of 25 miRNAs differed between control and MCT rats (p<0.05), including 15 miRNAs that were
increased in the MCT group (up to 1.9 fold), and 10 miRNAs that were decreased (up to -1.8 fold). These 25 miRNAs created a unique plasma signature that clearly distinguished control from MCT rats, as evidenced by distinct hierarchical clustering of the two groups (Figure 1A).

**MiRNA screening of plasma from PAH patients**

PCR arrays were used to measure the levels of 1066 miRNAs from the plasma of 4 treatment-naive idiopathic PAH patients and 3 healthy participants. The clinical characteristics of this derivation cohort are shown in Table 1. The plasma levels of 11 miRNAs were decreased in the IPAH group (up to -2.8 fold, p<0.05), while 14 miRNAs were increased (up to 3.4 fold). These miRNAs allowed for distinct clustering of the IPAH and healthy control subjects into separate groups (Figure 1B).

**Circulating miRNA-26a is decreased both in experimental and clinical PAH**

Three miRNAs (miR-365, -505, and -26a) were altered coordinately in plasma in both MCT rats and IPAH patients, none of which have previously been reported to be associated with PAH. The differential levels of these three miRNAs were evaluated in a second non-overlapping and broader human cohort comprised of 14 PAH patients and 13 healthy subjects (Table 1). Only miRNA-26a was significantly altered in the validation cohort, as confirmed by two different qPCR strategies (SYBR green-based standard qPCR: 1.8 fold down in PAH, p<0.01; TaqMan probe-based digital PCR: 1.7 fold down in PAH, p<0.001; Figure 1C). In addition, miR-26a showed strong performance as a biomarker for PAH with a large area under the corresponding ROC curve (AUC=0.85, p=0.002) (Figure 1D). The plasma levels of miR-26a also correlated directly with 6 minute walk distance (Pearson r = 0.64, p=0.01, Figure 1E).

**Altered miR-26a tissue expression mirrors the change in circulating levels**

To gain further insight into the potential factors that contribute to changes in circulating miRNA levels, we investigated miR-26a expression in MCT rat tissues. MiR-26a tissue expression was significantly reduced in the lung, and specifically in the right, but not left ventricle of the heart (Figure 1F). A significant inverse correlation was observed between miR-26a lung levels and the RV/(LV+S) mass ratio (Pearson r = -0.72, p=0.0003) and RVSP (r = -0.62, p=0.003).

**Circulating miRNA-26a is protected from endogenous plasma RNases primarily by protein complex binding**

Two complementary approaches were used to investigate the mechanism by which miR-26a is stabilized and transported within blood. First, nanovesicles ranging in size from ~50-150 nm were isolated from plasma with the ExoQuick™ exosome precipitation reagent. Although detectable in the resulting exosome-enriched pellet, miR-26a was nearly 10 fold more abundant in the protein-rich supernatant following precipitation (data not shown). Second, the
susceptibility of miR-26a to degradation by endogenous plasma ribonucleases was evaluated after treating plasma with a broad-spectrum protease (proteinase k) and/or membrane-disrupting surfactant (triton X-100). The level of miR-26a was significantly reduced (p<0.001) after treatment with proteinase k, but not triton X-100 (Figure 1G). Collectively, these results are consistent with a mechanism in which miR-26a is stabilized and transported within blood primarily by association with proteins rather than micro- or nanovesicles.

Conclusion

Here we identified circulating miR-26a as a novel biomarker candidate in PAH, which was concordantly reduced in the plasma of both MCT rats and treatment-naive IPAH patients as compared to healthy controls. The decrease in plasma miR-26a was validated in a separate, larger and more diverse cohort of PAH patients. Plasma miR-26a levels were positively correlated with 6MWD and showed diagnostic potential with a large area under the ROC curve. Moreover, miR-26a expression was significantly decreased in the lung and RV tissue of MCT rats, correlating with the hemodynamic abnormalities and RV remodeling in this model. Taken together, these results suggest that changes in circulating levels reflect intracellular regulation of miR-26a, which may contribute to disease activity underlying lung vascular and RV remodelling in PAH. Our future research will examine the potential cellular and extracellular roles of miR-26a using loss- and gain-of-function strategies in vascular cells and animal models of PH.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Derivation Cohort Healthy Control Subjects (n=3)</th>
<th>Derivation Cohort IPAH (n=4)</th>
<th>Validation Cohort Healthy Subjects (n=13)</th>
<th>Validation Cohort PAH (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>48 ± 8</td>
<td>52 ± 22</td>
<td>45 ± 13</td>
<td>58 ± 10†</td>
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<tr>
<td>Female, n (%)</td>
<td>2 (67)</td>
<td>3 (75)</td>
<td>9 (69)</td>
<td>8 (57)</td>
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<td>Cause of PAH, n (%)</td>
<td></td>
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<tr>
<td>Idiopathic</td>
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<td>0 (0)</td>
<td>N/A</td>
<td>8 (57)</td>
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<td>0 (0)</td>
<td>0 (0)</td>
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<td>0 (0)</td>
<td>8 (57)</td>
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<td>Class III</td>
<td>3 (75)</td>
<td>5 (36)</td>
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<td></td>
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<tr>
<td>Class IV</td>
<td>1 (25)</td>
<td>1 (7)</td>
<td></td>
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<td>PAH Medications, n (%)</td>
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<td>Diuretics</td>
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<td>Calcium channel blockers</td>
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<td>Endothelin Receptor blockers</td>
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<td>Hemodynamic parameters* (n)</td>
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<tr>
<td>mPAP, mm Hg</td>
<td>57 ± 5 (4)</td>
<td>48 ± 17 (12)</td>
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<td>PVR, dsc</td>
<td>914 ± 348 (4)</td>
<td>580 ± 147 (11)</td>
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<td>PCWP, mm Hg</td>
<td>12 ± 1 (4)</td>
<td>10 ± 5 (11)</td>
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* Mean ± STD
† p<0.01 (vs controls)

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References:


Figure 1. (A) Hierarchical clustering and heatmap showing relative levels of plasma miRNAs significantly altered between MCT and control rats (n=8/grp) (unadjusted p<0.05). Columns denote different rats, and rows denote different miRNAs. High relative plasma levels are denoted by red, and low plasma levels are denoted by green. Arrows denote miR-26a. (B) Heatmap showing the relative levels of miRNAs altered in the plasma of IPAH patients (n=4) versus healthy subjects (n=3). Columns denote different human participants, and rows denote different miRNAs. (C) Plasma miR-26a is significantly reduced in a second non-overlapping validation cohort of PAH patients and controls (n=13-14/grp). Left panel: SYBR green-based standard qPCR. Right panel: TaqMan probe-based digital PCR (performed independently in blinded fashion). ** p<0.01; *** p<0.001. (D) Receiver-operator characteristic (ROC) curve of hsa-miR-26a in validation cohort. AUC=area under the curve. (E) Plasma hsa-miR-26a level versus 6 min walk distance (6MWD) in PAH patients. Pearson correlation coefficient (r) shown. (F) Changes in tissue expression of rno-miR-26a in the MCT rat model as measured by RT-qPCR. * p<0.05. Data presented as mean ± SEM. (G) Mechanism of stability and transport of miR-26a in rat plasma. Rat plasma was treated with or without proteinase k (5mg/ml) and/or triton X-100 (1%) for 1 hr at 42°C, followed by RNA extraction and RT-qPCR measurement of miR-26a. *** p<0.001 versus untreated. Mean ± SEM (n=7). Adapted from ref. (3), © 2014 American Thoracic Society.
Ronald J. Oudiz, MD, FACP, FACC, FCCP is Professor of Medicine, David Geffen School of Medicine at UCLA and is the Director of the Pulmonary Hypertension Center and Faculty Cardiologist at the Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center in Southern California. Dr. Oudiz received his medical school training at the University of Southern California in Los Angeles, his Internal Medicine training at the University of California, San Diego, and his training in Cardiovascular Diseases at Harbor-UCLA Medical Center in Torrance, CA. He is board-certified in Internal Medicine and Cardiovascular Diseases. Dr. Oudiz is a past holder of scientific research awards from the American Heart Association and the National Institutes of Health. He has authored several papers in pulmonary hypertension and has presented his research at national and international seminars. Dr. Oudiz is the immediate past Editor-in-Chief of the scientific publication Advances in Pulmonary Hypertension. He has participated in several trials of innovative medical treatments for pulmonary hypertension (PH), many of which are still ongoing. Dr. Oudiz’s recent focus has been to describe the physiologic abnormalities that are caused by PH using measurements of lung gas exchange during exercise, and to study exercise rehabilitation as a treatment modality for patients with PH.
Dr. Harrison W. Farber is a Professor in the Department of Medicine and the Director of the Pulmonary Hypertension Center at Boston University.

He has focused on research into pulmonary arterial hypertension (PAH) and the clinical care of PAH patients for approximately 20 years. Dr. Farber has received numerous grants (both basic science and clinical) and has an extensive publication record in this area, including articles in peer-reviewed journals such as Circulation, New England Journal of Medicine, and Chest.

Dr. Farber serves on many panels for the development of clinical recommendations in PAH, has participated in large multicenter clinical trials, and is on the Steering Committee of the REVEAL Registry (Registry to Evaluate Early and Long Term PAH Disease Management), the largest registry of PAH patients ever created. His research interests include endothelial cell biology, in particular, the response of the pulmonary vasculature to injury.

After earning a medical degree at George Washington University School of Medicine, Dr. Farber completed an internship and residency at the Medical College of Virginia and a fellowship at Boston University.
*Biography*

**Adaani E. Frost, MD**
Professor of Medicine
Baylor College of Medicine
Houston, Texas

Dr. Adaani Frost is Professor of Medicine in the Pulmonary and Critical Care Section of Baylor College of Medicine, Houston, Texas. She undertook her postgraduate training in pulmonary and critical care, including a fellowship in lung transplantation in the Toronto Hospital System and McGill University. She was Medical Director of the Lung Transplant Program at both the Methodist Hospital and St. Luke’s Episcopal Hospital from 1990 to 2001 and developed the Pulmonary Hypertension and Advanced Lung Disease Service at Baylor. Currently, she is involved in clinical management and clinical research on patients with end stage lung disease, predominantly in pulmonary hypertension, and pulmonary fibrosis. Dr. Frost was on the Scientific Advisory Council of the Pulmonary Hypertension Association until 2009, is on the steering committee of REVEAL (a US-based registry of more than 3500 pulmonary hypertensive patients), has authored numerous papers on pulmonary hypertension, and is a participant in multiple new and ongoing studies in the treatment of pulmonary hypertension.
Mardi Gomberg-Maitland, MD, MSc, is Associate Professor of Medicine and Director of the Pulmonary Hypertension Program at the University of Chicago Medical Center in Chicago, Illinois. Dr. Gomberg-Maitland earned her undergraduate degree at Yale University, her medical degree at the Albert Einstein College of Medicine and completed a residency at New York Presbyterian Hospital-Weill/Cornell Medical Center and a fellowship at Mount Sinai Medical Center. She earned a Masters in Clinical Epidemiology at Harvard School of Public Health.

Dr. Gomberg-Maitland is an expert clinician and researcher in the field of pulmonary heart disease. In recent years, she has participated in dozens of multicenter, multinational research trials to explore new therapies for pulmonary hypertension. She is currently focusing on pulmonary arterial, pulmonary venous hypertension/diastolic dysfunction, and biomarker development.

A fellow of the American College of Cardiology, American College of Chest Physicians, and American Heart Association, and a member of the International Society of Heart and Lung Transplantation, American Thoracic Society, and Pulmonary Hypertension Association, Dr. Gomberg-Maitland has published numerous articles in peer-reviewed journals, including Circulation, Journal of the American College of Cardiology, Clinical Pharmacology and Therapeutics, Chest, European Respiratory Journal, and the American Journal of Respiratory and Critical Care Medicine.
Biography

MAUREEN D. MAYES, MD, MPH
Professor of Internal Medicine
Division of Rheumatology and Clinical Immunogenetics
The University of Texas Health Science Center at Houston
Houston, Texas

Dr. Mayes graduated from Eastern Virginia Medical School and completed her Internal Medicine training and Rheumatology fellowship at the Cleveland Clinic. She received a Master’s in Public Health (MPH) in Epidemiology from the University of Michigan School of Public Health. She joined the University of Texas – Houston Medical School faculty in 2002 and subsequently established the Scleroderma Clinic. Dr. Mayes is the recipient of many distinctions, awards and grants for the study and treatment of scleroderma. She is the author of over 100 published manuscripts, 19 reviews, 5 book chapters and 1 full length book. Her clinical interests include the treatment of scleroderma and its multiple complications. She participates in several multi-center, national trials of new agents for this disease. Her research interests include the identification of susceptibility genes and disease severity genes in scleroderma and related autoimmune diseases. She is currently the Principal Investigator of the NIH/NIAMS funded ‘Two-Stage Genome-Wide Association Study in Scleroderma’ that has the dual objectives to identify genes that influence disease susceptibility and severity, as well as to serve as a national resource to supply genetic material to other investigators to study this disease.
The ENTELLIGENCE Steering Committee

Biography

Evangelos D. Michelakis, MD

Professor of Medicine, Division of Cardiology
Vice Chair (Research) – Department of Medicine
Director, Pulmonary Hypertension Program
University of Alberta
Canada Research Chair in Applied Molecular and Mitochondrial Medicine
Edmonton, Alberta, Canada

Dr. Michelakis was born in Greece, where he went to Medical School at the University of Patras. He completed training in Vascular Biology, Internal Medicine, and Cardiology at the University of Texas (Galveston), Yale University, and the University of Minnesota. He joined the faculty of the University of Alberta in 1998, where he is now a full Professor and a Vice Chair (Research) in the Department of Medicine. Dr. Michelakis founded and has directed the Pulmonary Hypertension Program and clinic at the University of Alberta since 2001; this multidisciplinary clinic is open 5 days a week and treats patients referred from Alberta, Northern BC, Saskatchewan and Manitoba. He is also a vascular biologist and runs an active laboratory with several graduate students and technicians, focusing on the discovery of novel therapies for pulmonary hypertension. He is the Canada Research Chair in Applied Molecular and Mitochondrial Medicine and the Chair of the Cardiopulmonary, Critical Care, Perioperative and Resuscitation (3CPR) Council of the American Heart Association, and he serves on the editorial boards of both Circulation and Circulation Research. Recently, Dr. Michelakis has discovered intriguing similarities in the biology of pulmonary hypertension and cancer, which have led him into an exciting translational research program in cancer as well.
Harold I. Palevsky, MD, is a Professor of Medicine at the Perelman School of Medicine of the University of Pennsylvania. He is also Chief of the Pulmonary, Allergy and Critical Care Division and Director of the Pulmonary Vascular Disease Program at the Penn Presbyterian Medical Center in Philadelphia. The Pulmonary Vascular Disease Program is a multi-disciplinary program focusing on the diagnosis and treatment of pulmonary vascular disease, pulmonary arterial hypertension, and pulmonary thromboembolic disease, both acute and chronic.

Dr. Palevsky earned a medical degree from the Medical College of Virginia. He completed an internship and residency in internal medicine, and a fellowship in pulmonary and critical care medicine at the Hospital of the University of Pennsylvania, where he worked with Alfred P. Fishman, MD.

His clinical and research interests include unexplained dyspnea, pulmonary vascular disease, pulmonary hypertension, and thromboembolic disease. Dr. Palevsky has been published in numerous peer-reviewed journals, including the Annals of Internal Medicine, JAMA, and Circulation. He has been recognized as one of Philadelphia’s “Top Docs” and is included in national lists such as “The Best Doctors in America” and the “Guide to America’s Top Physicians.”
Richard M. Silver, MD
Distinguished University Professor of Medicine and Pediatrics
Director, Division of Rheumatology & Immunology
Medical University of South Carolina
Charleston, South Carolina

Dr. Richard Silver serves as Director of the Division of Rheumatology & Immunology at the Medical University of South Carolina (MUSC). He was born in Tennessee and graduated from the University of Tennessee–Knoxville. After graduating from Vanderbilt University School of Medicine in 1975, Dr. Silver completed training in Internal Medicine at the University of North Carolina-Chapel Hill, and then in Rheumatology at London’s Northwick Park Hospital and at the University of California-San Diego. He joined the faculty at MUSC in 1981, where he is a Distinguished University Professor of Medicine and Pediatrics. In 2007, MUSC’s Board of Trustees named him a “Master Teacher” and bestowed the University’s highest academic recognition, Distinguished University Professor. Also in 2007, the Scleroderma Foundation named him their “Doctor of the Year.” Dr. Silver’s major research interest is interstitial lung disease associated with systemic sclerosis.
Biography

JASON X-J YUAN, MD, PHD
Professor of Medicine
Associate Vice President for Translational Health Sciences
University of Arizona
Director, Division of Translational and Regenerative Medicine
Department of Medicine
University of Arizona College of Medicine
Tucson, Arizona

Dr. Jason Yuan is Professor of Medicine and Associate Vice President for Translational Health Sciences at the University of Arizona in Tucson, AZ. He is also Director of the Division of Translational and Regenerative Medicine in the Department of Medicine at the University of Arizona College of Medicine. Dr. Yuan received his medical school training at Suzhou Medical College (China), his PhD at Peking Union Medical College (China), and his postdoctoral training in the University of Maryland School of Medicine. His pulmonary vascular disease research propels the field on pathogenic roles of membrane receptors and ion channels and provides a new research direction for developing therapeutic approaches for the disease. Dr. Yuan is a Fellow of the American Heart Association, the American Association for the Advancement of Science, and the American Physiological Society. He is an elected Member of the American Society for Clinical Investigation and the Association of American Physicians. Dr. Yuan has served on many advisory committees and editorial boards, including Chair of the Respiratory Integrative Biology and Translational Research study section of the National Institutes of Health. He is currently Chair of the Pulmonary Circulation Assembly of the American Thoracic Society, Editor-in-Chief of the journal Pulmonary Circulation, and Associate Editor of the American Journal of Physiology-Cell Physiology.