Conference Abstracts

A record number of abstracts were submitted during PHA's 2014 International Conference and Scientific Sessions in June. The winning abstracts were presented as oral abstracts during the scientific sessions and are included in this issue of *Advances*.

Non-Biased Proteomics Discovery of Pulmonary Artery Hypertension Biomarkers Periostin and Matrix Metalloproteinase 9

Nies MK, Yang J, Fu Z, Everett AD
Johns Hopkins University, Baltimore, Maryland, USA

Background: Identification of a pulmonary hypertension (PH) biomarker would aid in risk stratification, non-invasive monitoring of therapeutic efficacy and even as a potential therapeutic target. Our aim was to identify PH biomarkers via proteomic analysis of PH and control lung, followed by complementary techniques to demonstrate alteration in PH lung and plasma.

Methods: Homogenized lung extract from the Pulmonary Hypertension Breakout Initiative (PHBI) biorepository from end stage PH patients (idiopathic PAH=5, PAH-associated with congenital heart disease=5, control= 5) was analyzed by non-biased, high resolution mass spectrometry (Orbitrap Elite). Selection of lead biomarkers was by biological feasibility and spectral counting with >2 or <0.5 fold change between PH and control. Lung western blot, IHC and ELISA were performed on PHBI homogenized lung, paraffin embedded lung and plasma, respectively, from adult PAH patients and controls. Data was analyzed by one way ANOVA and Kruskal Wallis.

Results: 860 non-redundant lung proteins were identified (APAH= 620, IPAH= 688, control=688; figure 1); 38 proteins were >2 fold up or <0.5 fold down in PH versus control lung. Periostin (IPAH 7.5 fold, APAH 9.5 fold) and matrix metalloproteinase 9 (MMP9) (IPAH and APAH <<0.5 fold) were identified as promising lead biomarkers. Western blot revealed elevated periostin while MMP9 decreased in PH versus control lung (figure 2a and 2b). Periostin IHC stained alveolar endothelial cells (figure 3). ELISA assay showed increased periostin in PH versus control plasma (n= 17, PH median 9738 ng/mL, control median 1433 ng/mL, p= 0.0502) (figure 4).

Conclusions: Periostin and MMP9 have reciprocal expression in PH lung. Periostin is predominately endothelial expressed, with circulating periostin a promising new PH biomarker. MMPs play a significant role in PH pathogenesis. Future studies will include larger sample sets, including pediatric patients, and evaluate additional potential PH biomarkers generated from proteomic lung analysis.

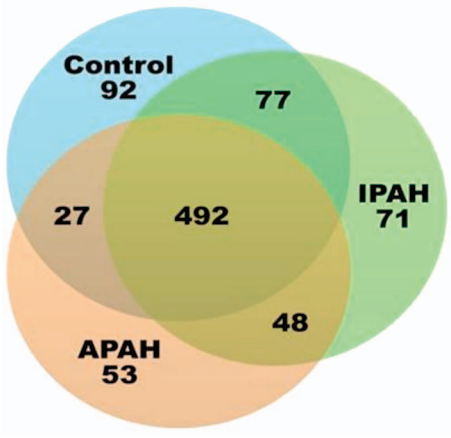

Figure 1: Lung Proteins

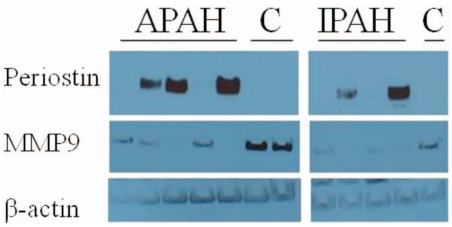

Figure 2A: Lung Western Blot

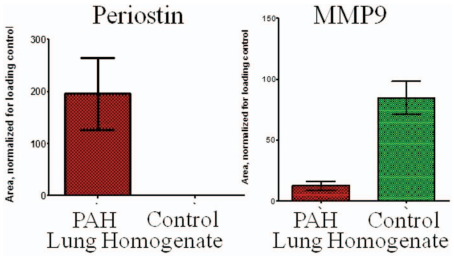

Figure 2B: Lung Western Blot Densitomery

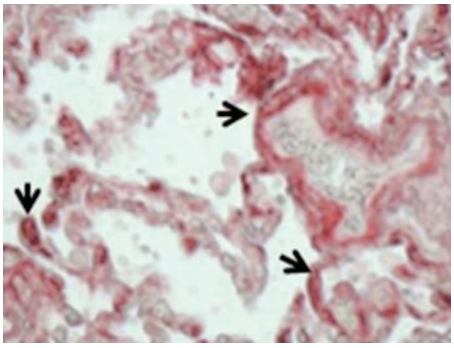

Figure 3: Periostin Immunohistochemistry

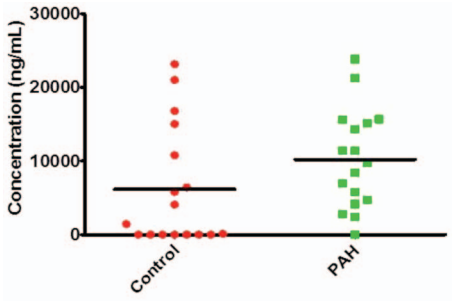

Figure 4: Plasma Periostin: PH vs control

Resistin-like Molecule Proteins Promote Pulmonary Vascular Endothelial Activation and Apoptosis: Cross Talk Between Vascular Endothelial and Smooth Muscle Cells

Yamaji-Kegan K,
Johns RA
Department of Anesthesiology and Critical Care Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Background: Pulmonary hypertension (PH) is a devastating disease of the pulmonary vasculature characterized by enhanced inflammation, vasoconstriction, pulmonary artery smooth muscle cell (PASMC) proliferation, and remodeling of small pulmonary arteries. Injury to endothelium and consequent wound repair cascades have been suggested to trigger pulmonary vascular remodeling in this disease. The relationship between injury to endothelium and disease pathogenesis in this disorder remains poorly understood. We and others have shown that in rodents, resistin-like molecule α (RELMα; also known as HIMF or FIZZ1) plays a critical role in the pathogenesis of lung inflammation and the development of PH. In this study, we dissected the mechanism by which RELMα/HIMF and its human homolog resistin (hResistin) induce pulmonary endothelial cell (EC) activation and apoptosis. We also examined the effect of conditioned media from RELMα/HIMF- or hResistin-treated ECs on PASMC proliferation.

Methods: We stimulated primary pulmonary microvascular EC from mouse or human with RELMα/HIMF or hResistin, respectively, and examined whether these RELM proteins induce apoptosis by TUNEL assay and analyzing apoptosis-related signaling. We also examined EC activation by quantifying the expression EC exocytosis components in response to hResistin. Lastly, we determined the effect of EC conditioned medium on PASMC proliferation and bone marrow derived (BMD) cell recruitment in response to RELM proteins.

Results: Both RELMα/HIMF and hResistin caused apoptosis in PMVEC. RELM α/HIMF-induced EC apoptosis is mediated by activation of p53 and caspase-3. hResistin treatment increased the expression of von Willebrand Factor and Angiopoietin-2 from PMVEC. These molecules are known as the EC exocytosis components that are released from EC in response to the stress/injury. EC conditioned medium treated with RELM proteins significantly enhanced PASMC proliferation and BMD cell recruitment as compared to non-treated EC conditioned control medium.

Conclusions: Our results suggest that RELMα/HIMF and hResistin induce EC activation and apoptosis, and these apoptotic EC lead the production of growth factors that stimulate PASMC proliferation. Thus, an EC apoptosis-SMC growth loop could result in the progression of pulmonary vascular remodeling in PH. The more detailed mechanisms by which PASMC growth factors and chemokines are regulated in pulmonary EC in response to RELM proteins is under investigation.

Right Ventriculo-Arterial Coupling in Patients with Pulmonary Arterial Hypertension Undergoing Rapid Dose Escalation of Treprostinil

Rischard F[1], Champion HC[2], Vanderpool R[2], Simon M[2], Jenkins I[3], Hansen L[1], Knoper S[1], Waxman A[4]
[1]Department of Pulmonary and Critical Care Medicine, University of Arizona, Tucson, Arizona, USA; [2]Pulmonary Vascular Disease Center, University of Pittsburgh, Pittsburgh, Pennsylvania, USA; [3]BIO5 Institute, University of Arizona, Tucson, Arizona, USA; [4]Pulmonary Vascular Disease Program, Brigham and Women's Hospital, Boston, Massachusetts, USA

Background: The mechanism of vasoactive therapy in pulmonary arterial hypertension (PAH) on ventriculo-arterial interaction is not well established. Therefore, we prospectively investigated the effect of treprostinil on pulmonary vascular elastance (Ea), right ventricular (RV) systolic (Ees) and coupling in PAH patients.

Methods and Results: Single-beat RV pressure-volume analysis was performed in 9 functional class IV PAH patients before and after rapid inpatient dose escalation, and after gradual outpatient dose increase of treprostinil. Data are presented as mean±SEM. Treprostinil dose was 12.8±0.46 ng/kg/min at discharge and 44.2±4.34 ng/kg/min by 3 months (mo.). Treprostinil was most consistently associated with a decrease in Ea (2.44±0.26 mmHg/ml baseline, 1.95±0.49 mmHg/ml discharge, and 1.38±0.28 mmHg/ml 3 mo. Ees was decreased slightly at discharge (1.62±0.35 mmHg/ml baseline vs. 1.49±0.3 mmHg/ml) and then reduced further by 3 mo. (0.85±0.18 mmHg/ml). The decrease in Ea, compared to Ees, was more pronounced at discharge, but relatively the same at 3 mo. leading to a coupling ratio of 0.68±0.15 baseline, 0.98±0.23 discharge, and 0.68±0.16 at 3 mo. Most patients demonstrate similar changes in Ea and Ees (both direction and magnitude) at discharge and 3 mo. Although Ees decreased at 3 mo., there was 906±530 mmHg*ml stroke work reserve with exercise and a 139±45 M increase in 6 minute walk distance vs. discharge. Both RV stroke work (RVSW) increased and work efficiency maintained at discharge and 3 mo. RV end-diastolic volumes (RV EDV) however remained elevated 241+28.2 ml baseline, 225+30.4 ml discharge, and 218+24.5 ml 3 mo.

Conclusions: Treprostinil primarily exerts its effects by lowering RV afterload (Ea) both acutely and at 3 mo. This effect governs the improvement in RV systolic pump failure and contractile reserve. Increases in RVSW are likely related to significant lowering of afterload and persistently high baseline preload dependent (heterometric) autoregulation.

Trends in Pediatric Inpatient Sildenafil Use in the United States, 2004-2013

Yung D[1], Tejtel KS[2]
[1]University of Washington, Bellevue, Washington, USA; [2]Baylor University, Waco, Texas, USA

Background: Since sildenafil became commercially available in 1998 and approved for adult pulmonary arterial hypertension (PAH) in 2005, reports of pediatric sildenafil use have increased. However, a black-box FDA warning was

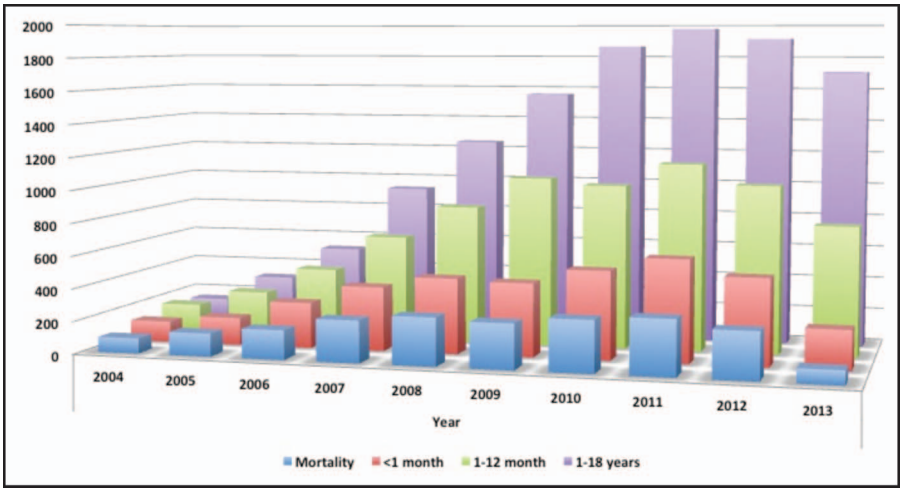

Figure 1: Number of patients with sildenafil use by age and year, plus mortality. The first use was reported in 2004.

given in the summer of 2012 based on results of a placebo-controlled trial in children ages 1-17. We sought to understand sildenafil use in hospitalized children over time.

Methods: The Pediatric Health Information System (PHIS) database was queried between January 2004 and June 2013 for sildenafil use in hospitalized patients with demographic, primary diagnosis and clinical data (Table 1, Figure 1).

Results: There were 23,164 hospitalized children who received at least one dose of sildenafil.

Conclusion: After the FDA warning against sildenafil use in children in 2012 there has been decrease in the use of sildenafil use in all age groups and an associated decrease in mortality.

Table 1. Clinical Data

	Number (%)
Primary Diagnosis	
Congenital Heart Disease	5589 (24)
Pulmonary hypertension	1624 (7)
Pneumonia (bacterial or viral)	1629 (7)
Prematurity or RDS	888 (4)
Gender, Male	12133 (52)
Length of stay 7 days or less	9816 (42)
Mechanical ventilation	13122 (57)
ECMO	1431 (6)
Readmission within one year	9748 (55)