Conference Abstracts

A record number of abstracts were submitted during PHA's 2016 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*.

PHD2 Deficiency in Endothelial Cells and Hematopoietic Cells Induces Obliterative Vascular Remodeling and Severe Pulmonary Arterial Hypertension Recapitulating Clinical PAH

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Purpose: The aim of this study is to determine the fundamental role of prolyl-4 hydroxylase 2 (PHD2) in regulating pulmonary vascular remodeling and the pathogenesis of severe pulmonary arterial hypertension (PAH).

Background: Vascular occlusion and complex plexiform lesions are hallmarks of the pathology of severe PAH in patients. However, mechanisms of obliterative vascular remodeling remain elusive, and hence current therapies have not targeted the fundamental disease-modifying mechanisms and result in only modest improvement in morbidity and mortality.

Methods: To gain insights into the role of PHD2 in the pathogenesis of PAH, Egln1 (encoding PHD2) floxed mice were bred with Tie2 promoter/enhancer-driven Cre transgenic mice to generate Egln1Tie2 mice. Egln1Tie2/Hif1aTie2 and Egln1Tie2/Hif2aTie2 double knockout mice were also generated. Right ventricular (RV) hemodynamic measurement and echocardiography were performed to evaluate the RV systolic pressure (RVSP), and cardiac size and function. RV to left ventricle (LV) plus septum (+S) ratio were also determined as an indicator of RV hypertrophy. Bone marrow cell transplantation was employed to determine the contribution of PHD2-deficient hematopoietic cells in PAH. Histology was used to quantify vascular remodeling. RNA sequencing, bioinformatics, and molecular analysis were carried out to define the molecular mechanisms of PAH in Egln1Tie2 mice.

Results: Mice with Tie2Cre-mediated disruption of (Egln1Tie2) in endothelial cells and hematopoietic cells exhibited spontaneous severe PAH (RVSP ranging from 60 to 90 mm Hg), with extensive pulmonary vascular remodeling including vascular occlusion and plexiform-like lesions resembling the hallmarks of the pathology of clinical PAH. As seen in idiopathic PAH patients, Egln1Tie2 mice exhibited unprecedented severe RV hypertrophy (RV/LV+S, 0.9±0.15) and failure and progressive mortality. Consistently, PHD2 expression was diminished in lung endothelial cells of obliterated pulmonary vessels in idiopathic PAH patients. Genetic deletions of both Egln1 and Hif1a or Egln1 and Hif2a identified hypoxia-inducible factor-2α (HIF-2α) as the critical mediator of severe PAH seen in Egln1Tie2 mice. We also observed altered expres-

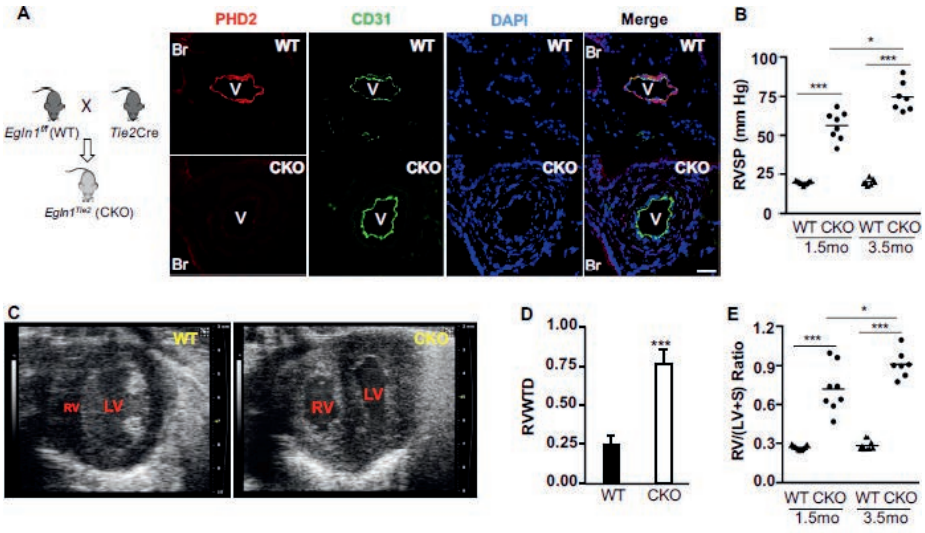


Fig 1. Figure 1. Spontaneous severe PAH and RV hypertrophy in *Egln1*^{Tie2} mice. (A) *Tie2*Cre-mediated disruption of *Egln1* in lung ECs. A diagram showing the strategy for generation of *Egln1*^{Tie2} mice. Representative micrographs of immunostaining showing EC-specific disruption of PHD2 in *Egln1*^{Tie2} mouse lungs . (B) Dramatic increase of RVSP in *Egln1*^{Tie2} mice. (C-D) Representative echocardiography showing an enlarged RV chamber and thickened RV wall (hypertrophy, C), and increased RV wall thickness diastole (RVWTD, D) in 3.5 mo old *Egln1*^{Tie2} mice. (E) Marked RV hypertrophy in *Egln1*^{Tie2} mice. *, $P < 0.05$; ***, $P < 0.001$ (Student's *t* test: D; *ANOVA* followed by Games-Howell post hoc analysis: B and E.

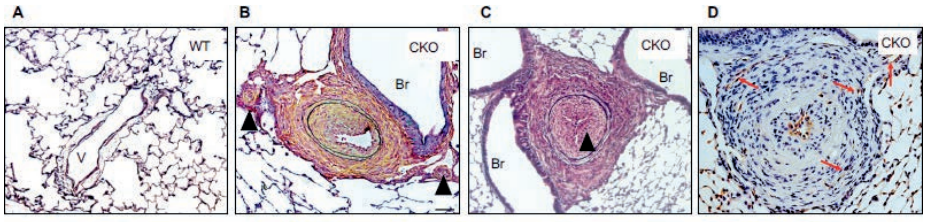


Fig 2. Occlusive pulmonary vascular remodeling in *Egln1*^{Tie2} mice. (A-C) Representative micrographs of Russel-Movat pentachrome staining demonstrating thickening of the intima, medial, and adventitial, occlusion of the large and small vessels (black arrowheads) in 3.5 mo old *Egln1*^{Tie2} mice (B, C) and WT mice (A). (D) Anti-CD31 immunohistochemistry showing multiple-channel lesions positive for the endothelial marker CD31 (arrows) in *Egln1*^{Tie2} mice.

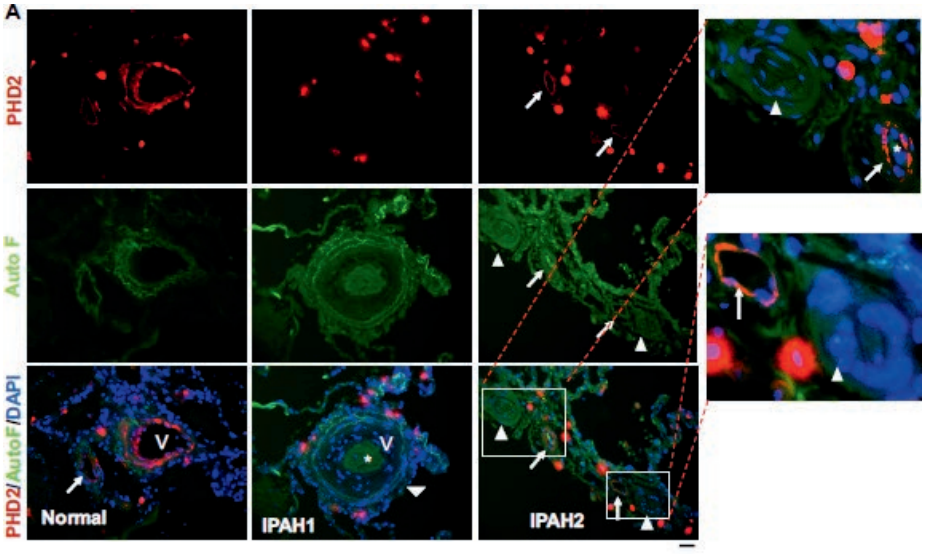
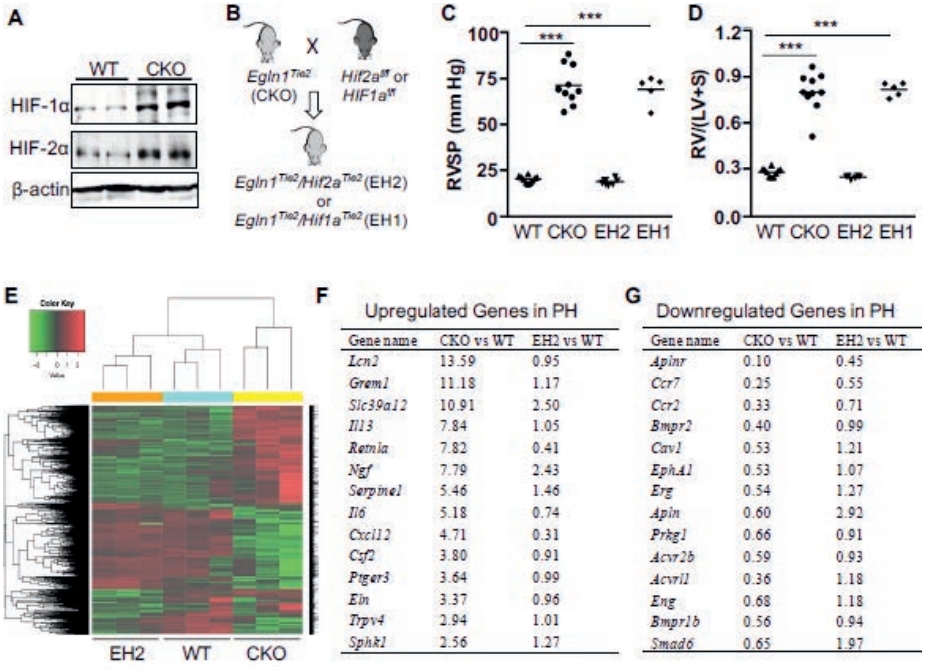


Fig 3. Diminished PHD2 expression in occlusive pulmonary vessels of IPAH patients. (**A**) Immunostaining demonstrating diminished PHD2 expression (red) in the lumen of occlusive vessels (arrowheads) of IPAH lungs. Nuclei were counterstained with DAPI (blue). Lung sections exhibited strong autofluorescence (AutoF) which helped to show the morphology. Arrows point to non-occlusive vessels; asterisk indicates blood cells. Scale bar, 50 μm.



F

Upregulated Genes in PH

Gene name	CKO vs WT	EH2 vs WT
Lcn2	13.59	0.95
Grem1	11.18	1.17
Slc39a12	10.91	2.50
Il13	7.84	1.05
Retnla	7.82	0.41
Ngf	7.79	2.43
Serpine1	5.46	1.46
Il6	5.18	0.74
Cxcl12	4.71	0.31
Csf2	3.80	0.91
Ptger3	3.64	0.99
Eln	3.37	0.96
Trpv4	2.94	1.01
Sphk1	2.56	1.27

G

Downregulated Genes in PH

Gene name	CKO vs WT	EH2 vs WT
Aplnr	0.10	0.45
Ccr7	0.25	0.55
Ccr2	0.33	0.71
Bmpr2	0.40	0.99
Cav1	0.53	1.21
EphA1	0.53	1.07
Erg	0.54	1.27
Apln	0.60	2.92
Prkg1	0.66	0.91
Acvr2b	0.59	0.93
Acvrl1	0.36	1.18
Eng	0.68	1.18
Bmpr1b	0.56	0.94
Smad6	0.65	1.97

Fig 4. Role of HIF-2α activation in *Egln1*^{Tie2} mice in mediating severe PAH. (A) Western blot showing stabilized HIF-1α and HIF-2α expression in *Egln1*^{Tie2} mouse lungs. (B-D) Genetic deletion of *HIF2α* but not *HIF1α* in *Egln1*^{Tie2} mice completely normalized RVSP (C) and inhibited RV hypertrophy (D) as evident by normalized RV/LV+S ratio at 2 months. (**E**) Representative heat map of RNA-seq analysis in WT, *Egln1*^{Tie2} (CKO) and *EH2* mouse lungs (n=3 mice/group). (**F-G**) RNA-seq analysis PH-causing genes in mouse lungs. ***, $P < 0.001$. *ANOVA* followed by Games-Howell post hoc analysis was used for statistical analysis.

sion of many pulmonary hypertension (PH)-causing genes in Egln1Tie2 lungs, which were normalized in Egln1Tie2/Hif2aTie2 lungs. Additionally, reconstitution of wild-type (WT) bone marrow cells in Egln1Tie2 chimeric mice attenuated PAH, whereas PHD2-deficient bone marrow cells failed to induce PAH in WT chimeric mice, demonstrating the essential role of PHD2 deficiency in endothelial cells in the development of PAH and PHD2 deficiency in bone marrow cells as an important contributor of the severity of the pathogenesis.

Conclusions: These studies defined an unexpected role of PHD2 deficiency in the mechanisms of severe PAH and identified the first genetically modified mouse model with irreversible obliterative vascular remodeling and pathophysiology recapitulating clinical PAH. Thus, targeting PHD2/HIF-2α signaling is a promising strategy to reverse vascular remodeling for treatment of severe PAH and promote survival.

A Selective TGFβ Ligand Trap Attenuates Pulmonary Arterial Hypertension

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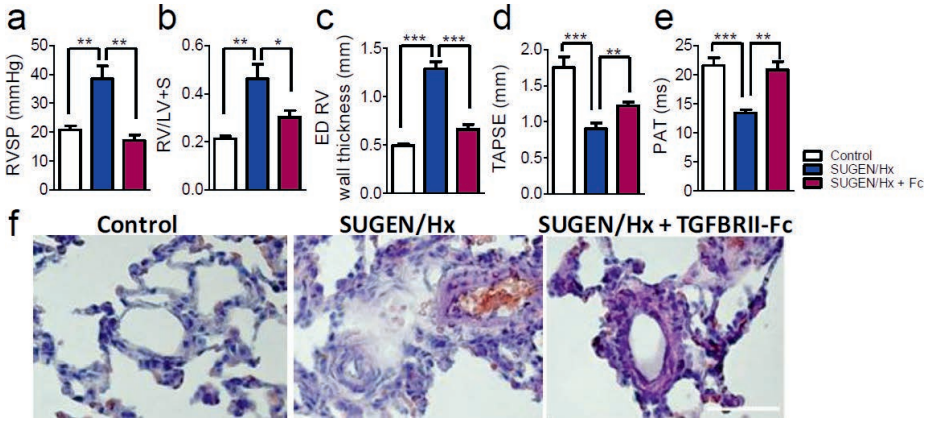
Purpose: We aim to test the impact of TGFBRII-Fc, a selective transforming growth factor-β (TGFβ)1/3 ligand trap, on experimental pulmonary arterial hypertension (PAH) and pulmonary vascular remodeling.

Background: Transforming growth factor-β ligands serve as critical regulators of development and tissue homeostasis, signaling via type I and type II serine-threonine kinase receptors to regulate broad transcriptional programs. Excessive TGFβ-mediated signaling is implicated in the pathogenesis of PAH, based in part on the ability of broad inhibitors of TGFβ/activin/growth and differentiation factor (GDF)/nodal receptors ALK4/5/7 to attenuate experimental PAH. While these inhibitors are effective and promising, their clinical



application is limited by cardiovascular and systemic toxicity. Also, these broad inhibition strategies do not delineate the specific contribution of TGFβ vs a multitude of other ligands. We tested the impact of TGFBRII-Fc, a selective TGFβ1/3 ligand trap, on experimental PAH and pulmonary vascular remodeling.

Methods: Signaling studies utilized cultured human pulmonary artery smooth muscle cells. PAH was studied in monocrotaline-treated Sprague-Dawley rats, SUGEN/hypoxia-treated Sprague-Dawley rats, and SUGEN/hypoxia-treated C57BL/6 mice. PAH, cardiac function, remodeling, and valve structure were assessed by ultrasound, invasive hemodynamic measurements, and histomorphometry.

Results: TGFBRII-Fc is an inhibitor of TGFβ1 and TGFβ3 but not TGFβ2 signaling. In vivo, treatment with TGFBRII-Fc attenuated SMAD2 phosphorylation, normalized expression of plasminogen activator inhibitor type 1 (PAI-1), and mitigated PAH and pulmonary vascular remodeling in monocrotaline-treated rats, SUGEN/hypoxia-treated rats, and SUGEN/hypoxia-treated mice. Administration of TGFBRII-Fc to monocrotaline-treated or SUGEN/hypoxia-treated rats with established PAH improved right ventricular systolic pressures, right ventricular function, and survival. Importantly, no cardiac structural or valvular abnormalities were observed following treatment with TGFBRII-Fc.

Conclusions: Our findings directly implicate TGFβ1/3 in the pathogenesis of PAH while demonstrating the efficacy and tolerability of selective TGFβ ligand blockade for improving hemodynamics, remodeling, and survival in PAH.

A Computer Simulation Model for Atrial Fenestration Sizing in Pulmonary Arterial Hypertension With Right Ventricular Failure

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Purpose: We sought to evaluate hemodynamics after atrial septostomy in pulmonary arterial hypertension (PAH) by means of a computer model. The specific objective was to derive the size of atrial fenestration to achieve predetermined hemodynamic parameters, for varying degrees of PAH.

Background: There have been significant advances in the medical management of PAH. Atrial septostomy continues to play a role in prolonging survival in severe PAH with right ventricular failure, as a bridge to transplantation or as an early intervention in combination with medical therapy. The procedure is associated with significant mortality due to severe hypoxemia, presumably due to inability to control the size of atrial communication and spontaneous closure rates after septostomy approach 50%. As the degree of atrial decompression will depend on the severity of PAH and atrial pressures, atrial fenestration of varying sizes may be required. The concept of atrial fenestration sizing for achieving desired hemodynamics has not been addressed.

Methods: The Aplysia Cardiovascular Lab (Aplysia Cardiovascular Lab, Aplysia Medical AB, Sweden) provides an overview of the complex real-time interactions between myocardial, valvular, and vascular function in the human cardiovascular system. The pulmonary vascular resistance (PVR), atrial, and ventricular compliances were altered in this model to simulate varying degrees of PAH. Creation of an atrial fenestration of diameters varying from 4 to 10 mm was simulated. The desired hemodynamics following the procedure were: right atrial pressure (RAP) <15 mm Hg, left atrial pressure (LAP) to not exceed 15 mm Hg, saturation (SaO2) >85%, and Qp:Qs ratio more than 0.75.

Results: There was greater fall in RAP compared to the increase in LAP for the same atrial fenestration size. An atrial communication of 8 mm diameter and 5 mm thickness caused an increase in LAP by 3.7 mm Hg, decreased RAP by 4.0 mm Hg, decreased Qp:Qs by 35%, and also caused a 13% decrease in SaO2 (to 80%) in a model of severe PAH (PVR, 10 Wood units [WU]). This trend persisted for more severe degrees of PAH, except that the SaO2 reached 72% (19% fall) when PVR was 17 WU. There were no significant hemodynamic differences related to the thickness of the atrial communication. The ideal diameter of atrial communication for the desired hemodynamics was calculated.

Conclusions: A 4 to 6 mm atrial fenestration would be ideal when PVR >10 WU. A fenestration size more than 8 mm is unlikely to be of benefit due to severe hypoxemia. Severe degrees of PAH would require fenestration of lesser diameters to prevent inadvertent hypoxemia and decompensation. The predetermination of the hemodynamics by means of a computer simulation model may be useful for interventional planning in PAH.

Evidence of Fatty Acid Metabolic Defects and Right Ventricular Lipotoxicity in Human Pulmonary Arterial Hypertension

Brittain EL, Talati M, Fessel JP, Zhu H, West J, Penner N, Funke M, Lewis

GD, Gerszten RE, Hamid R, Pugh ME, Austin ED, Newman JH, Hemnes AR

Background: The mechanisms of right ventricular (RV) failure in pulmonary arterial hypertension (PAH) are poorly understood. Abnormalities in fatty acid (FA) metabolism have been described in experimental models of PAH, but systemic and myocardial FA metabolism have not been studied in human PAH. We hypothesize the FA metabolic defects are present in human PAH and contribute to RV lipotoxicity.

Methods: We used human blood, RV tissue, and noninvasive imaging to characterize multiple steps in the FA metabolic pathway in PAH subjects and controls. Human plasma and RV long-chain acylcarnitines, ceramides, and carnitine palmitoyltransferase I activity were quantified using standard liquid chromatography/mass spectrometric methods. High-resolution respirometry was used to measure ex vivo RV oxygen consumption. Proton magnetic resonance spectroscopy was used to quantify in vivo myocardial lipid content.

Results: Circulating free FAs and long-chain acylcarnitines were elevated in PAH patients vs controls after adjusting for multiple comparisons (both $P<0.001$). Human RV long-chain FAs were increased and long-chain acylcarnitines were reduced nearly 100-fold in PAH vs controls ($P<0.001$). In vivo intramyocyte lipid content was 7-fold higher in human PAH vs controls (Figure 1; 1.4 ± 1.3 % triglyceride [TG] vs 0.22 ± 0.11 % TG, $P=0.02$). Ceramide, a mediator of lipotoxicity, was increased in human PAH RVs vs controls ($P=0.006$). Using an animal model of heritable PAH (BMPR2R899X), we demonstrated reduced FA oxidation via failure of palmitoylcarnitine to stimulate oxygen consumption in the PAH RV (Figure 2; $P<0.001$). Carnitine transporter gene expression and activity were similar between PAH and control RVs.

Conclusions: Abnormalities in FA metabolism can be detected in the blood and myocardium in human PAH and are associated with RV steatosis and lipotoxicity. Murine data suggest that lipotoxicity may arise from impaired FA oxidation. This study highlights specific metabolic pathways of potential therapeutic interest in PAH and establishes a tool to study their activity in vivo.

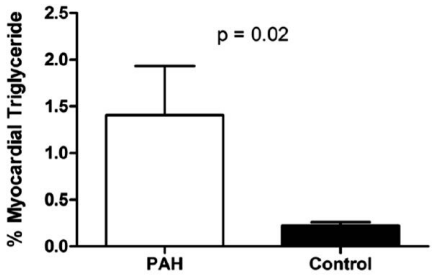

Figure 1. Increased *in vivo* myocardial triglyceride content in pulmonary arterial hypertension.

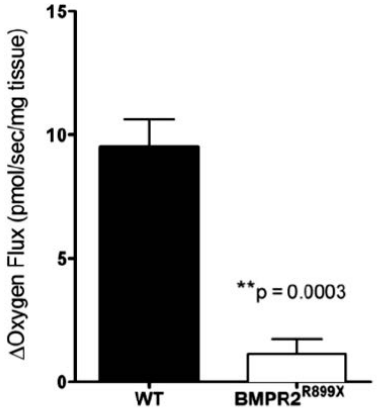

Figure 2. Failure of the PAH Right Ventricle to Augment Oxygen Consumption with Long-chain Acylcarnitine Supplementation