

## Hypertension Editors' Picks Pulmonary Hypertension

The Editors

Pulmonary artery hypertension (PAH) is a chronic and progressive disease characterized by persistent elevation of pulmonary arterial pressure caused by increased pulmonary vascular resistance and vascular remodeling. The resulting right ventricular hypertrophy leads ultimately to right heart dysfunction, organ failure, and death. Despite extensive research over the past 2 decades aimed at development of new therapies, PAH remains an incurable disease with high morbidity and mortality. Although systemic arterial hypertension and PAH are distinct clinical conditions, common pathophysiological processes underlie the two. Therefore, many important articles focused on the basic molecular mechanisms and signaling pathways responsible for PAH development appear in *Hypertension* each year. The goal, of course, is to identify novel treatment strategies for this devastating disease. Here, the Editors have assembled 17 full-length articles related to PAH, which were published in our Journal in 2014 and 2015. Potentially important signaling pathways highlighted include microRNAs,<sup>1,2</sup> myocardin-related transcription factor A,<sup>3</sup> kinins,<sup>4</sup> estradiol,<sup>5</sup> leukotrienes,<sup>6</sup> tumor necrosis factor- $\alpha$ ,<sup>7</sup> nuclear factor- $\kappa$ B,<sup>8</sup> oxidative stress,<sup>9,10</sup> TWIK-2 potassium channels,<sup>11</sup> and transient receptor potential channels.<sup>12</sup> Two articles<sup>13,14</sup> focus on possible adverse impacts of the sympathetic nervous system activation known to accompany PAH. Two studies were designed to improve the pharmacological management of PAH using novel drug delivery strategies.<sup>15,16</sup> And finally, 1 article<sup>17</sup> describes a much needed new animal model for investigating chronic thromboembolic pulmonary hypertension. It is clear that *Hypertension* continues to be a key venue for exciting new findings emerging from basic and clinical investigations into the pathophysiology of PAH.

### Development and Characterization of an Inducible Rat Model of Chronic Thromboembolic Pulmonary Hypertension<sup>17</sup>

#### Abstract

Chronic thromboembolic pulmonary hypertension (CTEPH) is an entity of PH that not only limits patients quality of life but also causes significant morbidity and mortality. The treatment of choice is pulmonary endarterectomy. However numerous patients do not qualify for pulmonary endarterectomy or present with residual vasculopathy post pulmonary endarterectomy and require specific vasodilator treatment. Currently, there is no available specific small animal model of CTEPH that could serve as tool to identify targetable molecular pathways and to test new treatment options. Thus, we generated and standardized a rat model that not only resembles functional and histological features of CTEPH but also emulates thrombi fibrosis. The pulmonary embolism protocol consisted of 3 sequential tail vein injections of fibrinogen/collagen-covered polystyrene microspheres combined with thrombin and administered to 10-week-old male Wistar rats. After the third embolism, rats developed characteristic features of CTEPH including elevated right ventricular systolic pressure, right ventricular cardiomyocyte hypertrophy, pulmonary artery remodeling, increased serum brain natriuretic peptide levels, thrombi fibrosis, and formation of pulmonary cellular-fibrotic lesions. The current animal model seems suitable for detailed study of CTEPH pathophysiology and permits preclinical testing of new pharmacological therapies against CTEPH

### Design, Characterization, and First-In-Human Study of the Vascular Actions of a Novel Biased Apelin Receptor Agonist<sup>15</sup>

#### Abstract

[Pyr(1)]apelin-13 is an endogenous vasodilator and inotrope but is downregulated in pulmonary hypertension and heart failure, making the apelin receptor an attractive therapeutic target. Agonists acting at the same G-protein-coupled receptor can be engineered to stabilize different conformational states and function as biased ligands, selectively stimulating either G-protein or  $\beta$ -arrestin pathways. We used molecular dynamics simulations of apelin/receptor interactions to design cyclic analogues and identified MM07 as a biased agonist. In  $\beta$ -arrestin and internalization assays (G-protein-independent), MM07 was 2 orders of magnitude less potent than [Pyr(1)]apelin-13. In a G-protein-dependent saphenous vein contraction assay, both peptides had comparable potency (pD<sub>2</sub>: [Pyr(1)]apelin-13 9.93 $\pm$ 0.24; MM07 9.54 $\pm$ 0.42) and maximum responses with a resulting bias for MM07 of  $\approx$ 350- to 1300-fold for the G-protein pathway. In rats, systemic infusions of MM07 (10–100 nmol) caused a dose-dependent increase in cardiac output that was significantly greater than the response to [Pyr(1)]apelin-13. Similarly, in human volunteers, MM07 produced a significant dose-dependent increase in forearm blood flow with a maximum dilatation double that is seen with [Pyr(1)]apelin-13. Additionally, repeated doses of MM07 produced reproducible increases in forearm blood flow. These responses are consistent with a more efficacious action of the biased agonist. In human hand vein, both peptides reversed an established norepinephrine constrictor response and significantly increased venous flow. Our results suggest that MM07 acting as a biased agonist at the apelin receptor can preferentially stimulate the G-protein pathway, which could translate to improved efficacy in the clinic by selectively stimulating vasodilatation and inotropic actions but avoiding activating detrimental  $\beta$ -arrestin-dependent pathways

## Megakaryocytic Leukemia 1 Directs a Histone H3 Lysine 4 Methyltransferase Complex to Regulate Hypoxic Pulmonary Hypertension<sup>3</sup>

### Abstract

Enhanced interaction between vascular endothelial cells and circulating leukocytes, as a result of transcriptional activation of cell adhesion molecules (CAM), helps establish a proinflammatory milieu contributing to the pathogenesis of chronic hypoxia-induced pulmonary hypertension. The molecular switch that dictates CAM transactivation is not clearly defined. Our goal was to determine the involvement of the transcriptional modulator megakaryocytic leukemia 1 (MKL1), also known as myocardin-related transcription factor A (MRTF-A), in CAM transactivation and the underlying mechanism. We report here that compared with wild-type littermates, MKL1/MRTF-A knockout mice were more resistant to the development of hypoxia-induced pulmonary hypertension when exposed to low oxygen pressure. Notably, CAM induction in knockout mice was significantly attenuated with a concomitant reduction of leukocyte adhesion. In cultured vascular endothelial cells, overexpression of MKL1/MRTF-A enhanced, whereas depletion of MKL1/MRTF-A dampened, hypoxia-induced CAM transactivation. In response to hypoxia, MKL1/MRTF-A formed a complex with NF- $\kappa$ B on the CAM promoters. Of interest, MKL1/MRTF-A was responsible for recruiting a histone H3 lysine 4 methyltransferase complex to the CAM promoters. Finally, endothelial-specific silencing of ASH2 and WDR5, 2 key components of the histone H3 lysine 4 methyltransferase complex, ameliorated hypoxia-induced pulmonary hypertension in mice. In conclusion, our data suggest that MKL1/MRTF-A, by coordinating key epigenetic alterations on CAM promoters, provides a critical link to hypoxia-induced endothelial malfunction and contributes to the pathogenesis of hypoxia-induced pulmonary hypertension.

## 17 $\beta$ -Estradiol Attenuates Conduit Pulmonary Artery Mechanical Property Changes With Pulmonary Arterial Hypertension<sup>5</sup>

### Abstract

Pulmonary arterial hypertension (PAH), a rapidly fatal vascular disease, strikes women more often than men. Paradoxically, female PAH patients have better prognosis and survival rates than males. The female sex hormone 17 $\beta$ -estradiol has been linked to the better outcome of PAH in females; however, the mechanisms by which 17 $\beta$ -estradiol alters PAH progression and outcomes remain unclear. Because proximal pulmonary arterial (PA) stiffness, one hallmark of PAH, is a powerful predictor of mortality and morbidity, we hypothesized that 17 $\beta$ -estradiol attenuates PAH-induced changes in mechanical properties in conduit proximal PAs, which imparts hemodynamic and energetic benefits to right ventricular function. To test this hypothesis, female mice were ovariectomized and treated with 17 $\beta$ -estradiol or placebo. PAH was induced in mice using SU5416 and chronic hypoxia. Extra-lobar left PAs were isolated and mechanically tested *ex vivo* to study both static and frequency-dependent mechanical behaviors in the presence or absence of smooth muscle cell activation. Our static mechanical test showed significant stiffening of large PAs with PAH ( $P < 0.05$ ). 17 $\beta$ -Estradiol restored PA compliance to control levels. The dynamic mechanical test demonstrated that 17 $\beta$ -estradiol protected the arterial wall from the PAH-induced frequency-dependent decline in dynamic stiffness and loss of viscosity with PAH ( $P < 0.05$ ). As demonstrated by the *in vivo* measurement of PA hemodynamics via right ventricular catheterization, modulation by 17 $\beta$ -estradiol of mechanical proximal PAs reduced pulsatile loading, which contributed to improved ventricular-vascular coupling. This study provides a mechanical mechanism for delayed disease progression and better outcome in female PAH patients and underscores the therapeutic potential of 17 $\beta$ -estradiol in PAH.

## miR-29a-3p Attenuates Hypoxic Pulmonary Hypertension by Inhibiting Pulmonary Adventitial Fibroblast Activation<sup>1</sup>

### Abstract

Activation of pulmonary adventitial fibroblasts plays a key role in the pulmonary vascular remodeling in hypoxic pulmonary hypertension. Previous studies showed that miRNAs participated in the regulation of fibroblast activation. This study explored the role of miR-29 in the activation of pulmonary adventitial fibroblasts and the therapeutic potential in hypoxic pulmonary hypertension. We found that hypoxia-induced pulmonary adventitial fibroblasts activation was accompanied with a drastic decrease of miR-29a-3p expression. Knockdown of hypoxia-inducible factor-1 $\alpha$  or Smad3 reversed the hypoxia-induced decrease of miR-29-3p in cultured pulmonary adventitial fibroblasts. *In vitro*, miR-29a-3p mimic inhibited the hypoxia-induced proliferation, migration, and secretion of pulmonary adventitial fibroblasts, suppressed the hypoxia-induced expression of  $\alpha$ -smooth muscle actin and extracellular matrix collagen in pulmonary adventitial fibroblasts; however, miR-29a-3p inhibitor mimicked the effect of hypoxia on the activation of pulmonary adventitial fibroblasts. Further studies revealed that preventative or therapeutic administration of miR-29a-3p significantly decreased pulmonary artery pressure and right ventricle hypertrophy index and ameliorated pulmonary vascular remodeling in hypoxic pulmonary hypertension rats. These findings suggest that miR-29a-3p regulates the activation and phenotype of pulmonary adventitial fibroblasts in hypoxia and has preventative and therapeutic potential in hypoxic pulmonary hypertension.

## Inhibition of Kinin B1 Receptors Attenuates Pulmonary Hypertension and Vascular Remodeling<sup>4</sup>

### Abstract

This study examined whether the kinin B1 receptor is involved in the pathogenesis of pulmonary hypertension, and whether its inhibition could reduce inflammation, pulmonary hypertension, vascular remodeling, and right heart dysfunction. Male Wistar rats underwent left pneumonectomy. Seven days later, the rats were injected subcutaneously with monocrotaline (60 mg/kg). The rats were then randomly assigned to receive treatment with vehicle or with BI113823 (a selective B1 receptor antagonist, 30 mg/kg, twice per day) via oral gavage from the day of monocrotaline injection to day 28. By day 28, BI113823-treated rats had significantly lower mean pulmonary artery pressure, less right ventricular hypertrophy, and pulmonary arterial neointimal formation than that of the vehicle-treated rats. Real-time polymerase chain reaction revealed that there was a significant increase in mRNA expression of B1 receptors in the lungs of monocrotaline-challenged pneumonectomized rats. Treatment with BI113823 significantly reduced macrophage recruitment, as measured via bronchoalveolar lavage. It also markedly reduced CD-68 positive macrophages and proliferating cell nuclear antigen positive cells in the perivascular areas, reduced expression of inducible nitric oxide synthase, matrix metalloproteinase 2 and 9, and B1 receptors compared with measurements in vehicle-treated rats. These findings demonstrate that kinin B1 receptors represent a novel therapeutic target for pulmonary arterial hypertension.

## Leukotriene B4 Activates Pulmonary Artery Adventitial Fibroblasts in Pulmonary Hypertension<sup>6</sup>

### Abstract

A recent study demonstrated a significant role for leukotriene B4 (LTB4) causing pulmonary vascular remodeling in pulmonary arterial hypertension. LTB4 was found to directly injure luminal endothelial cells and promote growth of the smooth muscle cell layer of pulmonary arterioles. The purpose of this study was to determine the effects of LTB4 on the pulmonary adventitial layer, largely composed of fibroblasts. Here, we demonstrate that LTB4 enhanced human pulmonary artery adventitial fibroblast proliferation, migration, and differentiation in a dose-dependent manner through its cognate G-protein-coupled receptor, BLT1. LTB4 activated human pulmonary artery adventitial fibroblast by upregulating p38 mitogen-activated protein kinase as well as Nox4-signaling pathways. In an autoimmune model of pulmonary hypertension, inhibition of these pathways blocked perivascular inflammation, decreased Nox4 expression, reduced reactive oxygen species production, reversed arteriolar adventitial fibroblast activation, and attenuated pulmonary hypertension development. This study uncovers a novel mechanism by which LTB4 further promotes pulmonary arterial hypertension pathogenesis, beyond its established effects on endothelial and smooth muscle cells, by activating adventitial fibroblasts.

## Effects of Intrinsic and Extrinsic Cardiac Nerves on Atrial Arrhythmia in Experimental Pulmonary Artery Hypertension<sup>13</sup>

### Abstract

Atrial arrhythmia, which includes atrial fibrillation (AF) and atrial flutter (AFL), is common in patients with pulmonary arterial hypertension (PAH), who often have increased sympathetic nerve activity. Here, we tested the hypothesis that autonomic nerves play important roles in vulnerability to AF/AFL in PAH. The atrial effective refractory period and AF/AFL inducibility at baseline and after anterior right ganglionated plexi ablation were determined during left stellate ganglion stimulation or left renal sympathetic nerve stimulation in beagle dogs with or without PAH. Then, sympathetic nerve,  $\beta$ -adrenergic receptor densities and connexin 43 expression in atrial tissues were assessed. The sum of the window of vulnerability to AF/AFL was increased in the right atrium compared with the left atrium at baseline in the PAH dogs but not in the controls. The atrial effective refractory period dispersion was increased in the control dogs, but not in the PAH dogs, during left stellate ganglion stimulation. The voltage thresholds for inducing AF/AFL during anterior right ganglionated plexi stimulation were lower in the PAH dogs than in the controls. The AF/AFL inducibility was suppressed after ablation of the anterior right ganglionated plexi in the PAH dogs. The PAH dogs had higher sympathetic nerve and  $\beta$ 1-adrenergic receptor densities, increased levels of nonphosphorylated connexin 43, and heterogeneous connexin 43 expression in the right atrium when compared with the control dogs. The anterior right ganglionated plexi play important roles in the induction of AF/AFL. AF/AFL induction was associated with right atrium substrate remodeling in dogs with PAH.

## AAV Delivery of Tumor Necrosis Factor- $\alpha$ Short Hairpin RNA Attenuates Cold-Induced Pulmonary Hypertension and Pulmonary Arterial Remodeling<sup>7</sup>

### Abstract

Cold temperatures are associated with increased mortality and morbidity of cardiovascular and pulmonary disease. Cold exposure causes lung inflammation, pulmonary hypertension (PH), and right ventricle hypertrophy, but there is no effective therapy because of unknown mechanism. Here, we investigated whether RNAi silencing of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) decreases cold-induced macrophage infiltration, PH, and pulmonary arterial (PA) remodeling. We found for the first time that continuous cold exposure (5.0°C) increased TNF- $\alpha$  expression and macrophage infiltration in the lungs and PAs right before elevation of right ventricle systolic pressure. The *in vivo* RNAi silencing of TNF- $\alpha$  was achieved by intravenous delivery of recombinant AAV-2 carrying TNF- $\alpha$  short hairpin siRNA (TNFshRNA) 24 hours before cold exposure. Cold exposure for 8 weeks significantly increased right ventricle pressure compared with the warm controls (40.19 $\pm$ 4.9 versus 22.9 $\pm$ 1.1 mmHg), indicating that cold exposure caused PH. Cold exposure increased TNF- $\alpha$ , interleukin-6, and phosphodiesterase-1C protein expression in the lungs and PAs and increased lung macrophage infiltration. Notably, TNFshRNA prevented the cold-induced increases in TNF- $\alpha$ , interleukin-6, and phosphodiesterase-1C protein expression, abolished lung macrophage infiltration, and attenuated PH (26.28 $\pm$ 1.6 mmHg), PA remodeling, and right ventricle hypertrophy. PA smooth muscle cells isolated from cold-exposed animals showed increased intracellular superoxide levels and cell proliferation along with decreased intracellular cGMP. These cold-induced changes were prevented by TNFshRNA. In conclusions, upregulation of TNF- $\alpha$  played a critical role in the pathogenesis of cold-induced PH by promoting pulmonary macrophage infiltration and inflammation. AAV delivery of TNFshRNA may be an effective therapeutic approach for cold-induced PH and PA remodeling.

## Oxidative Stress Is Associated With Increased Pulmonary Artery Systolic Pressure in Humans<sup>9</sup>

### Abstract

Oxidative stress contributes to the development of pulmonary hypertension in experimental models, but this association in humans is unknown. We investigated the relationship between pulmonary artery systolic pressure measured by echocardiography and plasma aminothiols oxidative stress markers, with the hypothesis that oxidative stress will be higher in those with pulmonary hypertension. A group of 347 patients aged 65 $\pm$ 12 years from the Emory Cardiovascular Biobank underwent echocardiographic assessment of left ventricular ejection fraction and pulmonary artery systolic pressure. Plasma aminothiols, cysteine, its oxidized form, cystine, glutathione, and its oxidized disulphide were measured and the redox potentials (Eh) of cysteine/cystine and glutathione/oxidized glutathione couples were calculated. Non-normally distributed variables were log transformed (Ln). Univariate predictors of pulmonary artery systolic pressure included age ( $P<0.001$ ), sex ( $P=0.002$ ), mitral regurgitation ( $P<0.001$ ), left ventricular ejection fraction ( $P<0.001$ ), left atrial size ( $P<0.001$ ), diabetes mellitus ( $P=0.03$ ), plasma Ln cystine ( $\beta=9.53$ ;  $P<0.001$ ), Ln glutathione ( $\beta=-5.4$ ;  $P=0.002$ ), and Eh glutathione ( $\beta=0.21$ ;  $P=0.001$ ). A multivariate linear regression model adjusting for all confounding variables demonstrated that Ln cystine ( $\beta=6.56$ ;  $P=0.007$ ), mitral regurgitation ( $\beta=4.52$ ;  $P<0.001$ ), statin use ( $\beta=-3.39$ ;  $P=0.03$ ), left ventricular ejection fraction ( $\beta=-0.26$ ;  $P=0.003$ ), and age ( $\beta=0.17$ ;  $P=0.003$ ) were independent predictors of pulmonary artery systolic pressure. For each 1% increase in plasma cystine, pulmonary artery systolic pressure increased by 16%. This association persisted in the subgroup with preserved left ventricular ejection fraction ( $\geq 50\%$ ) and no significant mitral regurgitation. Whether treatment of oxidative stress will improve pulmonary hypertension requires further study.

## Inhibition of Nuclear Factor- $\kappa$ B in the Lungs Prevents Monocrotaline-Induced Pulmonary Hypertension in Mice<sup>8</sup>

### Abstract

Pulmonary arterial hypertension (PAH) is a devastating cardiopulmonary disorder with significant morbidity and mortality in patients with various lung and heart diseases. PAH is characterized by vascular obstruction which leads to a sustained increased pulmonary vascular resistance, vascular remodeling, and right ventricular hypertrophy and failure. Limited PAH therapies indicate that novel approaches are urgently needed for the treatment of PAH. Nuclear factor- $\kappa$ B (NF- $\kappa$ B) has been shown to play an important role in different cardiac pathologies; however, the role of NF- $\kappa$ B remains limited in the setting of PAH. Here, we investigated whether NF- $\kappa$ B inhibition in the lungs using Club (Clara) cell-10 promoter driving I $\kappa$ B $\alpha$  mutant had any effect in monocrotaline (MCT)-induced PAH mouse model. Our data revealed that MCT-induced PAH and right ventricular hypertrophy were associated with NF- $\kappa$ B activation, inflammatory response, and altered expression of bone morphogenetic protein receptor 2, inhibitor of differentiation, and Notch-3 signaling molecules in wild-type mice; and all these alterations were prevented in I $\kappa$ B $\alpha$  mutant mice treated with MCT. Moreover, endothelial cell apoptosis and endothelial-to-mesenchymal transition occurred in the lungs of MCT-treated wild-type mice and were restored in I $\kappa$ B $\alpha$  mutant+MCT mice, indicating an association with NF- $\kappa$ B signaling. In lung microvascular endothelial cells, I $\kappa$ B $\alpha$  (AA) mutant plasmid restored the decreased bone morphogenetic protein receptor 2 protein level and reversed the endothelial-to-mesenchymal transition process induced by transforming growth factor- $\beta$ 1. We conclude that NF- $\kappa$ B regulates bone morphogenetic protein receptor 2-inhibitor of differentiation-Notch-3 axis genes and the subsequent endothelial cell apoptosis and endothelial-to-mesenchymal transition events in the lungs, providing new mechanistic information about MCT-induced PAH and right ventricular hypertrophy.

## Cervical Ganglion Block Attenuates the Progression of Pulmonary Hypertension via Nitric Oxide and Arginase Pathways<sup>14</sup>

### Abstract

It has been recognized that the sympathetic nervous system is activated in pulmonary arterial hypertension (PAH), and abnormal sympathetic hyperactivity leads to worsening of PAH via endothelial dysfunction. The purpose of this study was to examine whether sympathetic ganglion block (SGB) can treat PAH by increasing the availability of nitric oxide. PAH was induced in rats by 50 mg/kg of subcutaneous monocrotaline. After 2 weeks, daily injections of ropivacaine into the left superior cervical ganglion were repeated for 14 days (monocrotaline-SGB group). Monocrotaline group received sham SGB with saline, whereas control group received saline instead of monocrotaline. PAH was evident in monocrotaline group, with right ventricular systolic pressures (47±4 mmHg) that were higher than those of controls (17±2 mmHg), whereas SGB significantly attenuated monocrotaline-induced PAH (35±4 mmHg). The right/left ventricular mass ratios exhibited similar changes to those seen with right ventricular pressures. Heart rate variability showed significantly higher sympathetic activity in the monocrotaline group. Microscopy revealed a higher proportion of muscular arteries with thicker medial walls in the monocrotaline group, which was attenuated by SGB. Monocrotaline induced arginase hyperactivity, which was in turn decreased by SGB-induced endothelial nitric oxide synthase activation. SGB restored monocrotaline-induced hypoactivity of superoxide dismutase. In conclusion, SGB could suppress PAH and the remodeling of pulmonary arteries via inactivation of arginase and reciprocal elevation of nitric oxide bioavailability, thus attenuating disproportionate hyperactivation of the sympathetic nervous system.

## TWIK-2 Channel Deficiency Leads to Pulmonary Hypertension Through a rho-Kinase-Mediated Process<sup>11</sup>

### Abstract

TWIK-2 (KCNK6) is a member of the 2-pore domain (K2P) family of potassium channels, which are highly expressed in the vascular system. We tested the hypothesis that TWIK-2 deficiency leads to pulmonary hypertension. TWIK-2 knockout mice and their wildtype littermates at 8 weeks of age had similar mean right ventricular systolic pressures (24±3 and 21±3 mmHg, respectively.) Significantly, by 20 weeks of age, the mean right ventricular systolic pressures in TWIK-2 knockout mice increased to 35±3 mmHg ( $P\leq 0.036$ ), whereas mean right ventricular systolic pressures in wildtype littermates remained at 22±3 mmHg. Elevated mean right ventricular systolic pressures in the TWIK-2 knockout mice was accompanied by pulmonary vascular remodeling as determined by a 25% increase in the cross-sectional area of the vessels occupied by the vessel wall. Additionally, secondary branches of the pulmonary artery from 20-week-old TWIK-2 knockout mice showed an enhanced contractile response to U46619 (10(-6) moles/L), a thromboxane A2 mimetic, which was completely abolished with the Rho-kinase inhibitor, Y27632 (10(-6) and 10(-5) moles/L). Treatment of TWIK-2 knockout mice with the Rho-kinase inhibitor, fasudil, in the drinking water for 12 weeks, abolished the development of pulmonary hypertension and attenuated the vessel remodeling. We concluded that mice deficient in the TWIK-2 channel develop pulmonary hypertension between 8 and 20 weeks of age through a mechanism involving Rho-kinase. Our results suggest that downregulation of TWIK-2 in the pulmonary vasculature may be an underlying mechanism in the development of pulmonary hypertension.

## Increased Reactive Oxygen Species, Metabolic Maladaptation, and Autophagy Contribute to Pulmonary Arterial Hypertension-Induced Ventricular Hypertrophy and Diastolic Heart Failure<sup>10</sup>

### Abstract

Pulmonary arterial hypertension (PAH) is a debilitating and deadly disease with no known cure. Heart failure is a major comorbidity and a common cause of the premature death of patients with PAH. Increased asymmetrical right ventricular hypertrophy and septal wall thickening compress the left ventricular cavity and elicit diastolic heart failure. In this study, we used the Sugen5416/hypoxia/normoxia-induced PAH rat to determine whether altered pyridine nucleotide signaling in the failing heart contributes to (1) increased oxidative stress, (2) changes in metabolic phenotype, (3) autophagy, and (4) the PAH-induced failure. We found that increased reactive oxygen species, metabolic maladaptation, and autophagy contributed to the pathogenesis of right ventricular remodeling and hypertrophy that lead to left ventricular diastolic dysfunction. In addition, arterial elastance increased in PAH rats. Glucose-6-phosphate dehydrogenase is a major source of pyridine molecule (nicotinamide adenine dinucleotide phosphate), which is a substrate for nicotinamide adenine dinucleotide phosphate oxidases in the heart. Dehydroepiandrosterone, a 17-ketosteroid that reduces pulmonary hypertension and right ventricular hypertrophy, inhibited glucose-6-phosphate dehydrogenase, decreased oxidative stress, increased glucose oxidation and acetyl-coA, and reduced autophagy in the hearts of PAH rats. It also decreased arterial stiffness and improved left ventricular diastolic function. These findings demonstrate that pyridine nucleotide signaling, at least partly, mediates PAH-induced diastolic heart failure, and that reduction of glucose-6-phosphate dehydrogenase-derived nicotinamide adenine dinucleotide phosphate is beneficial to improve left ventricle diastolic function.

## Oral Delivery of Angiotensin-Converting Enzyme 2 and Angiotensin (1–7) Bioencapsulated in Plant Cells Attenuates Pulmonary Hypertension<sup>16</sup>

### Abstract

Emerging evidences indicate that diminished activity of the vasoprotective axis of the renin-angiotensin system, constituting angiotensin-converting enzyme 2 (ACE2) and its enzymatic product, angiotensin-(1–7) [Ang-(1–7)] contribute to the pathogenesis of pulmonary hypertension (PH). However, long-term repetitive delivery of ACE2 or Ang-(1–7) would require enhanced protein stability and ease of administration to improve patient compliance. Chloroplast expression of therapeutic proteins enables their bioencapsulation within plant cells to protect against gastric enzymatic degradation and facilitates long-term storage at room temperature. Besides, fusion to a transmucosal carrier helps effective systemic absorption from the intestine on oral delivery. We hypothesized that bioencapsulating ACE2 or Ang-(1–7) fused to the cholera nontoxin B subunit would enable development of an oral delivery system that is effective in treating PH. PH was induced in male Sprague Dawley rats by monocrotaline administration. Subset of animals was simultaneously treated with bioencapsulated ACE2 or Ang-(1–7) (prevention protocol). In a separate set of experiments, drug treatment was initiated after 2 weeks of PH induction (reversal protocol). Oral feeding of rats with bioencapsulated ACE2 or Ang-(1–7) prevented the development of monocrotaline-induced PH and improved associated cardiopulmonary pathophysiology. Furthermore, in the reversal protocol, oral ACE2 or Ang-(1–7) treatment significantly arrested disease progression, along with improvement in right heart function, and decrease in pulmonary vessel wall thickness. In addition, a combination therapy with ACE2 and Ang-(1–7) augmented the beneficial effects against monocrotaline-induced lung injury. Our study provides proof-of-concept for a novel low-cost oral ACE2 or Ang-(1–7) delivery system using transplastomic technology for pulmonary disease therapeutics.

## Endothelial Apoptosis in Pulmonary Hypertension Is Controlled by a microRNA/Programmed Cell Death 4/Caspase-3 Axis<sup>2</sup>

### Abstract

Pulmonary endothelial cell apoptosis is a transient, yet defining pathogenic event integral to the onset of many pulmonary vascular diseases such as pulmonary hypertension (PH). However, there is a paucity of information concerning the molecular pathway(s) that control pulmonary arterial endothelial cell apoptosis. Here, we introduce a molecular axis that when functionally active seems to induce pulmonary arterial endothelial cell apoptosis *in vitro* and PH *in vivo*. In response to apoptotic stimuli, human pulmonary arterial endothelial cells exhibited robust induction of a programmed cell death 4 (PDCD4)/caspase-3/apoptotic pathway that was reversible by direct PDCD4 silencing. Indirectly, this pathway was also repressed by delivery of a microRNA-21 mimic. *In vivo*, genetic deletion of microRNA-21 in mice (miR-21<sup>-/-</sup>) mice resulted in functional activation of the PDCD4/caspase-3 axis in the pulmonary tissues, leading to the onset of progressive PH. Conversely, microRNA-21-overexpressing mice (CAG-microRNA-21 mice) exhibited reduced PDCD4 expression in pulmonary tissues and were partially resistant to PH in response to chronic hypoxia plus SU 5416 injury. Furthermore, direct PDCD4 knockout in mice (PDCD4<sup>-/-</sup>) mice potently blocked pulmonary caspase-3 activation and the development of chronic hypoxia plus SU 5416 PH, confirming its importance in disease onset. Broadly, these findings support the existence of a microRNA-21-responsive PDCD4/caspase-3 pathway in the pulmonary tissues that when active serves to promote endothelial apoptosis *in vitro* and PH *in vivo*.

## Classical Transient Receptor Potential 1 and 6 Contribute to Hypoxic Pulmonary Hypertension Through Differential Regulation of Pulmonary Vascular Functions<sup>12</sup>

### Abstract

Hypoxic pulmonary hypertension is characterized by increased vascular tone, altered vasoreactivity, and vascular remodeling, which are associated with alterations in Ca<sup>2+</sup> homeostasis in pulmonary arterial smooth muscle cells. We have previously shown that classical transient receptor potential 1 and 6 (TRPC1 and TRPC6) are upregulated in pulmonary arteries (PAs) of chronic hypoxic rats, but it is unclear whether these channels are essential for the development of pulmonary hypertension. Here we found that pulmonary hypertension was suppressed in TRPC1 and TRPC6 knockout (Trpc1<sup>-/-</sup> and Trpc6<sup>-/-</sup>) mice compared with wild-type after exposure to 10% O<sub>2</sub> for 1 and 3 weeks. Muscularization of pulmonary microvessels was inhibited, but rarefaction was unaltered in hypoxic Trpc1<sup>-/-</sup> and Trpc6<sup>-/-</sup> mice. Small PAs of normoxic wild-type mice exhibited vasomotor tone, which was significantly enhanced by chronic hypoxia. Similar vasomotor tone was found in normoxic Trpc1<sup>-/-</sup> PAs, but the hypoxia-induced enhancement was blunted. In contrast, there was minimal vascular tone in normoxic Trpc6<sup>-/-</sup> PAs, but the hypoxia-enhanced tone was preserved. Chronic hypoxia caused significant increase in serotonin-induced vasoconstriction; the augmented vasoreactivity was attenuated in Trpc1<sup>-/-</sup> and eliminated in Trpc6<sup>-/-</sup> PAs. Moreover, the effects of 3-week hypoxia on pulmonary arterial pressure, right ventricular hypertrophy, and muscularization of microvessels were further suppressed in TRPC1-TRPC6 double-knockout mice. Our results, therefore, provide clear evidence that TRPC1 and TRPC6 participate differentially in various pathophysiological processes, and that the presence of TRPC1 and TRPC6 is essential for the full development of hypoxic pulmonary hypertension in the mouse model.

## Acknowledgments

We thank Dr Gregory D. Fink for contributing the introduction to this article.

## Disclosures

None.

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## *Hypertension* Editors' Picks: Pulmonary Hypertension

*Hypertension*. 2016;68:e47-e53; originally published online August 1, 2016;

doi: 10.1161/HYPERTENSIONAHA.116.08096

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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