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A Novel Insight into the Mechanism of Pulmonary Hypertension Involving Caveolin-1 Deficiency and eNOS Activation

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Abstract

Severe pulmonary hypertension (PH) is characterized by a progressive increase in pulmonary vascular resistance and vascular remodeling leading to right heart failure and early death. Our recent studies employing the novel mouse model with genetic deletions of caveolin-1 (Cav1) and eNOS (NOS3) have demonstrated that persistent eNOS activation in Cav1^{-/-} lungs results in tyrosine nitration of protein kinase G (PKG) and impairment of its activity, which thereby induces PH. The finding of eNOS activation and PKG nitration concomitant with Cav1 deficiency was recapitulated in lungs from patients with idiopathic pulmonary arterial hypertension. These data suggest targeting PKG nitration has potential value for the treatment of PH. Here, we will review the current knowledge about Cav1-regulated eNOS activity and its fundamental role in the pathogenesis of PH.

Introduction

Pulmonary hypertension (PH) is defined as a mean pulmonary arterial pressure greater than 25 mmHg at rest or 30 mm Hg with exercise (Farber and Loscalzo 2004). PH of different etiologies share several features: endothelial dysfunction, worsening vasoconstriction, remodeling of pulmonary microvessels, and intravascular thrombosis (Farber and Loscalzo 2004, Rabinovitch 1997, Rubin 1997). These changes typically result in increased medial thickness, microvascular occlusion, and formation of plexiform lesions, all of which contribute to the mechanism of increased pulmonary vascular resistance (PVR) and PH. As the molecular mechanisms responsible for pulmonary vascular remodeling and vasoconstriction remain elusive, there are limited options available for the prevention and treatment of progressive PH, including idiopathic pulmonary arterial hypertension (IPAH) (Farber 2008; Puri et al. 2007). IPAH is the most severe form of PH, which without treatment leads to right heart failure and premature death (Farber and Loscalzo 2004, Rabinovitch 1997, Rubin 1997, Runo and Loyd 2003). Studies have described mutations of bone morphogenetic protein receptor type II (BMPRII) in patients with familial PAH and IPAH (Lane et al. 2000; Machado et al. 2006). Given that only 10-15% of these individuals go on to develop severe disease (Humbert et al. 2006, Machado et al. 2006), other genetic and environmental factors are likely to be important. One important factor may be oxidative/nitrative stress (Bowers et al. 2004, Hoshikawa et al. 2001, Nozik-Grayck and Stenmark 2007). Tissue hypoxia, ischemia, and inflammation all

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contribute to the production of reactive oxygen species (ROS) in the lung tissue of patients with severe PH (Bowers et al. 2004, Hoshikawa et al. 2001). The reaction between ROS and nitric oxide (NO) results in not only low bioavailability of NO but also formation of peroxynitrite and resultant nitrative stress (Beckman et al. 1990, Hurst 2002). Although increased oxidative/nitrative stress has been demonstrated in lung tissues of patients with severe PH, including IPAH (Bowers et al. 2004, Cracowski et al. 2001), the mechanistic role of oxidative/nitrative stress in the pathogenesis of PH has not been fully elucidated. Here, we discuss the critical role of eNOS activation secondary to caveolin-1 (Cav1) deficiency in the pathogenesis of PH through tyrosine nitration-mediated impairment of protein kinase G (PKG) activity.

Caveolin-1 regulation of eNOS activity

Caveolae, 50-100 nm vesicular invaginations of the cell plasma membrane, have emerged as the site of the important events at the plasma membrane such as vesicular trafficking as well as signal transduction (Galbiati et al. 2001, Rothberg et al. 1992). Three isoforms of caveolins (Cav1, Cav2 and Cav3), the structural proteins of caveolae, have been identified. Caveolins are shown to act as scaffolding proteins to concentrate and orchestrate many signaling molecules, including eNOS, receptor and non-receptor tyrosine kinase receptors, G protein-coupled receptors, GTPase, and components of the mitogen-activated protein kinases, and regulate their function (Cohen et al. 2004, Galbiati et al. 2001, Rothberg et al. 1992). Cav1, a 22-kDa protein of 178 amino acids, is highly expressed in adipocytes, endothelial cells, fibroblasts and type I pneumocytes. Cav1 expression is sufficient and necessary to drive the formation of morphologically identifiable caveolae in these cells (Cohen et al. 2004). Through its scaffolding domain (residues 82-101), Cav1 acts as an anchor holding various proteins within caveolae and regulates their function (Cohen et al. 2004, Galbiati et al. 2001, Rothberg et al. 1992).

Endothelial cell-derived nitric oxide (NO) is a critical mediator of cardiovascular homeostasis regulating multiple physiological and pathophysiological processes including vascular tone, vascular remodeling, platelet aggregation, and angiogenesis (Ignarro et al. 1999). The enzyme activity of eNOS isoform (also called NOS3, predominantly expressed in endothelial cells) is regulated by fatty acid modification, post-translational phosphorylation, and interaction with effector molecules including Cav1 and heat shock protein 90 (Hsp90). eNOS is mostly targeted to caveolae in the plasma membrane through interaction with Cav1 in endothelial cells (Feron et al. 1996), which is dynamically regulated by Ca^{2+} -calmodulin (Michel et al. 1997). The primary binding region of Cav1 with eNOS is within the scaffolding domain (amino acids 60-101) (Ju et al. 1997). Amount evidences have demonstrated that Cav1 binding to eNOS is a crucial negative regulator of eNOS activity. *In vitro* studies have shown that interaction of eNOS with GST-Cav1 fusion proteins significantly inhibits its enzyme activity (Ju et al. 1997). A membrane permeable chimeric peptide containing a cellular internalization sequence fused to the Cav1 scaffolding domain (residues 82-101) has been demonstrated as a potent inhibitor of eNOS in mice and reduces inflammation (Bucci et al. 2000). Furthermore, genetic deletion of *Cav1* in mice results in a marked increase in plasma NO levels (Zhao et al. 2002) and enhanced eNOS activation in vessels from *Cav1*^{-/-} mice (Drab et al. 2001). We recently also demonstrate that loss of Cav1 results in 3-fold increase of eNOS-derived NO production in lung tissues from *Cav1*^{-/-} mice (Zhao et al. 2009). However, genetic deletion of eNOS completely blocks the increase of total NO production induced by Cav1 deficiency in lung tissues (Zhao et al. 2009). Consistent with previous studies, the interaction of eNOS with Hsp90, a positive regulator of eNOS activity is enhanced in lung tissues from *Cav1*^{-/-} mice (Zhao et al. 2009). In addition, abnormal Cav1-eNOS interaction is associated with various pathophysiological conditions such as atherosclerosis. It has been shown that hypercholesterolemia-induced decrease in NO production is ascribed to enhanced interaction

of Cav1 and eNOS (Feron et al. 1999). These data clearly demonstrate Cav1 as a crucial negative regulator of eNOS activity.

Pathogenic role of caveolin-1 deficiency in PH

The critical role of Cav1 deficiency in the pathogenesis of PH was first identified by Zhao and colleagues (Zhao et al. 2002). Cav1-deficient mice exhibit pulmonary hypertension and right ventricle hypertrophy. Hemodynamic measurements also reveal a significant increase of right ventricular contractility and diastolic function at baseline in *Cav1*^{-/-} mice compared to age- and gender-matched wild-type mice. Our recent studies further demonstrate that *Cav1*^{-/-} lungs exhibit increased pulmonary vascular resistance associated with pulmonary vascular remodeling including increased medial thickness and muscularization of distal pulmonary vessels (an underlying feature of pulmonary vascular remodeling in PH) (Zhao et al. 2009). Consistent with the role of Cav1 in regulating cell proliferation, *Cav1*^{-/-} lungs exhibit hypercellularity and alveolar septal thickening (Drab et al. 2001, Razani et al. 2001, Zhao et al. 2009). Maniatis et al. also identify a marked decrease of pulmonary artery density and defective pulmonary artery filling in *Cav1*^{-/-} lungs (Maniatis et al. 2008). These studies with *Cav1*^{-/-} mice provide direct evidence of the critical role of Cav1 deficiency in the pathogenesis of PH.

Cav1 deficiency is also seen in rat models of PH including monocrotaline-induced PH (Mathew et al. 2004) and SU5416/hypoxia-induced PH (Achcar et al. 2006). Cav1 (mainly the endothelial cell-restricted isoform Cav1 α) expression is decreased in rat lungs as early as 48 h and reaches nadir (70% reduction) at 2 weeks post-monocrotaline challenge (Mathew et al. 2004). Intriguingly, the progressive reduction of Cav1 expression is mainly identified in the intimal layer of the pulmonary arteries, not the pulmonary veins. Decreased Cav1 expression and associated hyperactivation of STAT3 and ERK1/2 signaling and cell proliferation are confirmed in pulmonary endothelial cell cultures treated with monocrotaline-pyrrole (Mathew et al. 2004). In the rat model of severe PH induced by a single s.c. injection of the VEGF receptor inhibitor SU5416 and subsequent 3 weeks exposure of chronic hypoxia, Cav1 expression is selectively decreased or diminished in the complex cellular arterial lesions (Achcar et al. 2006). Interestingly, the cell-permeable Cav1 inhibitory peptide has been shown effective to prevent the development of monocrotaline-induced PH in rats (Jasmin et al. 2006).

Decreased expression of Cav1 has also been demonstrated in lungs of patients with severe PH. Achcar et al. showed absent or decreased expression of Cav1 in plexiform lesions and in some muscularized precapillary arterioles in lung tissues from patients with severe PH although Cav1 expression in total lung lysates is not significantly changed (Achcar et al. 2006). Patel et al. identify decreased expression of Cav1 in total lung lysates and pulmonary vascular endothelial cells in lungs tissues from IPAH patients. In contrast, Cav1 expression is, however, increased in pulmonary vascular smooth muscle cells in lung tissues from IPAH patients (Patel et al. 2007). Our recent studies also demonstrate a marked decrease of Cav1 expression in total lung lysates from IPAH patients (Zhao et al. 2009). Taken together, these data strongly support the concept that Cav1 is a critical regulator of the pulmonary vascular function in animals and humans.

Causal role of eNOS activation secondary to Cav1 deficiency in the pathogenesis of PH

To determine the consequences of chronic activation of eNOS in *Cav1*^{-/-} lungs, our recent studies have employed a mouse model with genetic deletions of both *Cav1* and *NOS3* (DKO) (Zhao et al. 2009). Surprisingly, DKO mice don't develop pulmonary hypertension. In contrast

to *Cav1*^{-/-} mice, DKO mice have the same right ventricular systolic pressure (RVSP) as WT mice. The ratio of right weight /left ventricle plus septum weight is normal in DKO mice and PVR in DKO mice is reduced to the WT level. The defects in pulmonary vasculatures in *Cav1*^{-/-} mice are also corrected in DKO mice. DKO lungs exhibit normal alveolar-capillary structure and vessel wall thickness in contrast to *Cav1*^{-/-} lungs. Muscularization, an indicator of vascular remodeling, of distal pulmonary arteries is inhibited in DKO lungs compared to *Cav1*^{-/-} lungs. In *Cav1*^{-/-} lungs, pulmonary vascular remodeling is associated with hyperactivation of ERK signaling and downregulation of p21^{Cip1}, a cyclin-dependent kinase inhibitor. These cell cycle progression-related signaling events are normalized in DKO lung tissues as seen in WT lungs. These data provide unequivocal evidence that chronic activation of eNOS secondary to Cav1 deficiency is a critical factor in the mechanism of PH. Consistent with our observations, Wunderlich et al. also show that L-NAME inhibition of NOS prevents adverse lung remodeling and PH in *Cav1*^{-/-} mice (Wunderlich et al. 2008). However, previous studies have shown that increased production of NO in eNOS transgenic mice prevents the increase in RVSP, lung vascular remodeling, and right ventricular hypertrophy induced by chronic hypoxia (Ozaki et al. 2001), and cell-based eNOS gene transfer also inhibits monocrotaline-induced PH in rats (Zhao et al. 2005).

Mechanistic role of PKG nitration in the pathogenesis of PH

Our recent studies provide additional insights into the molecular basis of eNOS activation-induced PH in *Cav1*^{-/-} mice. Given that NO reacts with superoxide to form the damaging reactive oxygen species peroxynitrite, which modifies proteins and may interfere with their function through tyrosine nitration, we have detected a significant increase in nitrotyrosine expression, a surrogate measure of peroxynitrite in *Cav1*^{-/-} lungs. Prominent nitrotyrosine immunostaining is also evident in *Cav1*^{-/-} pulmonary vasculature, indicating peroxynitrite formation in *Cav1*^{-/-} lungs. Accordingly, PKG, the downstream target of NO signaling responsible for vasorelaxation is tyrosine-nitrated by peroxynitrite and its kinase activity is impaired in *Cav1*^{-/-} lungs. However, genetic deletion of eNOS blocks peroxynitrite formation and resultant PKG nitration, and thereby restores PKG activity in DKO lungs. In primary culture of human pulmonary arterial smooth muscle cells, treatment with 3-morpholiniosydnonimine (SIN-1), a superoxide and NO donor that forms peroxynitrite simultaneously, induces PKG nitration and impairs its kinase activity. Site-directed mutagenesis and *in vitro* PKG kinase activity assays have identified tyrosine residues 345 and 549 in the kinase domain of human PKG-1 α as the potential target tyrosines responsible for impaired PKG activity following nitration by peroxynitrite in *Cav1*^{-/-} lungs. PKG nitration and resultant impairment of its kinase activity in the pulmonary vasculature under hypoxia condition have also been reported by other investigators (Negash et al. 2007). In contrast to our observation in *Cav1*^{-/-} lungs, the hypoxia-induced tyrosine nitration of PKG in these studies is eNOS-independent.

To address the causal role of tyrosine nitration-mediated impairment of PKG activity in the development of PH in *Cav1*^{-/-} mice, a series of experiments including blocking peroxynitrite formation and overexpressing PKG have been performed (Zhao et al. 2009). To block peroxynitrite formation, *Cav1*^{-/-} mice are treated with either L-NAME to inhibit NOS or manganese (III) tetrakis (1-methyl-4-pyridyl) porphyrin pentachloride (MnTMPyP, a superoxide dismutase mimetic) to scavenge superoxide. Following 5 to 6 weeks treatment of either L-NAME or MnTMPyP, the pulmonary hypertensive phenotypes in *Cav1*^{-/-} lungs including increased RVSP and pulmonary vascular remodeling are reversed. These data suggest that formation of peroxynitrite and resultant protein nitration are required for the development of PH in *Cav1*^{-/-} mice. To determine nitrate stress-induced PH in *Cav1*^{-/-} mice is mediated by tyrosine nitration-induced impairment of PKG activity, PKG-1 is overexpressed in *Cav1*^{-/-} lungs through recombinant adenovirus-mediated transduction.

Restoration of PKG activity results in reduction of RVSP and PVR in *Cav1*^{-/-} mice. Taken together, these data strongly support the concept that nitrative stress-induced PH in *Cav1*^{-/-} mice is mediated by impaired PKG kinase activity through tyrosine nitration.

To address the pathophysiological relevance of Cav1-regulated eNOS activity in the mechanism of increased PVR and pulmonary vascular remodeling in patients with PH, we have examined Cav1 expression, eNOS activity and PKG nitration in lung tissues from IPAH patients (Zhao et al. 2009). We observed increased eNOS activity and PKG nitration concomitant with decreased Cav1 expression in lung tissues from IPAH patients compared with normal lungs in the absence of marked changes of eNOS and PKG expression. These findings are in agreement with the studies in *Cav1*^{-/-} mouse lungs. Previous observations that decreased Cav1 expression in IPAH lungs is mainly in the plexiform lesions (Achcar et al. 2006) and selectively in endothelial cells (Patel et al. 2007), and that eNOS is robustly expressed in the plexiform lesions of IPAH lungs (Mason et al. 1998) support the concept that eNOS (predominantly expressed in endothelial cells) activation secondary to Cav1 deficiency plays a causal role in the mechanism of PH in mice and humans through PKG nitration and resultant impairment of its kinase activity (Figure 1).

Conclusions and future perspectives

Severe PH (including IPAH) is a fatal disease characterized by a progressive increase in PVR and vascular remodeling leading to right heart failure and early death. Despite recent advances in our understanding of the mechanisms and genetic determinants of PH, there are limited options available for the prevention and treatment of severe PH. Prominent oxidative/nitrative stress is a hallmark of the pathology of severe PH. Our recent studies have now provided a mechanistic insight into the critical role of oxidative/nitrative stress in the pathogenesis of PH in mice. We have demonstrated the crucial role of chronic eNOS activation secondary to Cav1 deficiency in the mechanism of PH and provide evidence that PKG nitration induces PH. These key observations in mice were recapitulated in lung tissue from IPAH patients. To further establish the causal role of PKG nitration in the pathogenesis of PH, it would be interesting to determine whether overexpression of PKG mutants resistant to tyrosine nitration in the pulmonary vasculature will be more efficient to reverse the hypertensive pulmonary phenotypes in *Cav1*^{-/-} mice and in other experimental animal models of PH. Second, it remains unclear what are the source(s) of superoxide in *Cav1*^{-/-} lungs. Although Wunderlich et al. have shown evidence of eNOS uncoupling as a potential source of ROS production in *Cav1*^{-/-} lungs, eNOS uncoupling alone doesn't cause PH in other studies. In addition, it also remains unknown in which cell type(s) Cav1 deficiency induces PH. Future studies directed to address these questions will strengthen our understanding of the mechanism of progressive PH. Importantly, future efforts may be directed to identify signaling pathways regulating oxidative/nitrative stress and determine the correlation of oxidative/nitrative stress and the severity of PH. These new studies will provide us with a comprehensive understanding of the molecular basis of oxidative/nitrative stress-induced progression of PH, and thereby identify potential novel therapeutic approaches targeting the underlying signaling pathways for the prevention and treatment of progressive PH. Recently, homologous transplantation of stem/progenitor cells has shown efficacy in treatment of severe PH. Stem/progenitor cells-based eNOS overexpression is currently under clinical trial for treatment of PH. On the basis of our recent studies, it would be interesting to see whether stem/progenitor cells-based gene therapy targeting oxidative/nitrative stress is a more efficacious approach for the treatment of severe PH.

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References

- Achcar RO, Demura Y, Rai PR, et al. Loss of caveolin and heme oxygenase expression in severe pulmonary hypertension. *Chest* 2006;129:696–705. [PubMed: 16537870]
- Beckman JS, Beckman TW, Chen J, et al. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc Natl Acad Sci U S A* 1990;87:1620–1624. [PubMed: 2154753]
- Bowers R, Cool C, Murphy RC, et al. Oxidative stress in severe pulmonary hypertension. *Am J Respir Crit Care Med* 2004;169:764–769. [PubMed: 14701708]
- Bucci M, Gratton JP, Rudic RD, et al. In vivo delivery of the caveolin-1 scaffolding domain inhibits nitric oxide synthesis and reduces inflammation. *Nat Med* 2000;6:1362–1367. [PubMed: 11100121]
- Cohen AW, Hnasko R, Schubert W, Lisanti MP. Role of caveolae and caveolins in health and disease. *Physiol Rev* 2004;84:1341–1379. [PubMed: 15383654]
- Cracowski JL, Cracowski C, Bessard G, et al. Increased lipid peroxidation in patients with pulmonary hypertension. *Am J Respir Crit Care Med* 2001;164:1038–42. [PubMed: 11587993]
- Drab M, Verkade P, Elger M, et al. Loss of caveolae, vascular dysfunction, and pulmonary defects in caveolin-1 gene-disrupted mice. *Science* 2001;293:2449–2452. [PubMed: 11498544]
- Farber HW. The status of pulmonary arterial hypertension in 2008. *Circulation* 2008;117:2966–2968. [PubMed: 18541751]
- Farber HW, Loscalzo J. Pulmonary arterial hypertension. *N Engl J Med* 2004;351:1655–1665. [PubMed: 15483284]
- Feron O, Belhassen L, Kobzik L, et al. Endothelial nitric oxide synthase targeting to caveolae: Specific interactions with caveolin isoforms in cardiac myocytes and endothelial cells. *J Biol Chem* 1996;271:22810–22814. [PubMed: 8798458]
- Feron O, Dessy C, Moniotte S, et al. Hypercholesterolemia decreases nitric oxide production by promoting the interaction of caveolin and endothelial nitric oxide synthase. *J Clin Invest* 1999;103:897–905. [PubMed: 10079111]
- Galbiati F, Razani B, Lisanti MP. Emerging themes in lipid rafts and caveolae. *Cell* 2001;106:403–411. [PubMed: 11525727]
- Geraci MW, Moore M, Gesell T, et al. Gene expression patterns in the lungs of patients with primary pulmonary hypertension: a gene microarray analysis. *Circ Res* 2001;88:555–562. [PubMed: 11282888]
- Hoshikawa Y, Ono S, Suzuki S, et al. Generation of oxidative stress contributes to the development of pulmonary hypertension induced by hypoxia. *J Appl Physiol* 2001;90:1299–12306. [PubMed: 11247927]
- Humbert M, Sitbon O, Chaouat A, et al. Pulmonary arterial hypertension in France: results from a national registry. *Am J Respir Crit Care Med* 2006;173:1023–30. [PubMed: 16456139]
- Hurst JK. Whence nitrotyrosine? *J Clin Invest* 2002;109:1287–1289. [PubMed: 12021243]
- Ignarro LJ, Cirino G, Casini A, Napoli C. Nitric oxide as a signaling molecule in the vascular system: an overview. *J Cardiovasc Pharmacol* 1999;34:879–886. [PubMed: 10598133]
- Jasmin JF, Mercier I, Dupuis J, et al. Short-term administration of a cell-permeable caveolin-1 peptide prevents the development of monocrotaline-induced pulmonary hypertension and right ventricular hypertrophy. *Circulation* 2006;114:912–920. [PubMed: 16940204]
- Ju H, Zou R, Venema VJ, Venema RC. Direct interaction of endothelial nitric-oxide synthase and caveolin-1 inhibits synthase activity. *J Biol Chem* 1997;272:18522–18525. [PubMed: 9228013]
- Lane KB, Machado RD, Pauciulo MW, et al. Heterozygous germline mutations in BMPR2, encoding a TGF-beta receptor, cause familial primary pulmonary hypertension. *Nat Genet* 2000;26:81–84. [PubMed: 10973254]

- Machado RD, Aldred MA, James V, et al. Mutations of the TGF-beta type II receptor BMPR2 in pulmonary arterial hypertension. *Hum Mutat* 2006;27:121–132. [PubMed: 16429395]
- Maniatis NA, Shinin V, Schraufnagel DE, et al. Increased pulmonary vascular resistance and defective pulmonary artery filling in caveolin-1^{-/-} mice. *Am J Physiol Lung Cell Mol Physiol* 2008;294:L865–L873. [PubMed: 18192592]
- Mason NA, Springall DR, Burke M, et al. High expression of endothelial nitric oxide synthase in plexiform lesions of pulmonary hypertension. *J Pathol* 1998;185:313–318. [PubMed: 9771486]
- Mathew R, Huang J, Shah M, et al. Disruption of endothelial-cell caveolin-1alpha/raft scaffolding during development of monocrotaline-induced pulmonary hypertension. *Circulation* 2004;110:1499–1506. [PubMed: 15353500]
- Michel JB, Feron O, Sacks D, Michel T. Reciprocal regulation of endothelial nitric-oxide synthase by Ca²⁺-calmodulin and caveolin. *J Biol Chem* 1997;272:15583–15586. [PubMed: 9188442]
- Negash S, Gao Y, Zhou W, et al. Regulation of cGMP-dependent protein kinase-mediated vasodilation by hypoxia-induced reactive species in ovine fetal pulmonary veins. *Am J Physiol Lung Cell Mol Physiol* 2007;293:L1012–L1020. [PubMed: 17616649]
- Nozik-Grayck E, Stenmark KR. Role of reactive oxygen species in chronic hypoxia-induced pulmonary hypertension and vascular remodeling. *Adv Exp Med Biol* 2007;618:101–112. [PubMed: 18269191]
- Ozaki M, Kawashima S, Yamashita T, et al. Reduced hypoxic pulmonary vascular remodeling by nitric oxide from the endothelium. *Hypertension* 2001;37:322–327. [PubMed: 11230292]
- Patel HH, Zhang S, Murray F, et al. Increased smooth muscle cell expression of caveolin-1 and caveolae contribute to the pathophysiology of idiopathic pulmonary arterial hypertension. *Faseb J* 2007;21:2970–2979. [PubMed: 17470567]
- Puri A, McGoon MD, Kushwaha SS. Pulmonary arterial hypertension: current therapeutic strategies. *Nat Clin Pract Cardiovasc Med* 2007;4:319–329. [PubMed: 17522721]
- Rabinovitch M. Pulmonary hypertension: updating a mysterious disease. *Cardiovasc Res* 1997;34:268–272. [PubMed: 9205539]
- Razani B, Engelman JA, Wang XB, et al. Caveolin-1 null mice are viable but show evidence of hyperproliferative and vascular abnormalities. *J Biol Chem* 2001;276:38121–38138. [PubMed: 11457855]
- Rothberg KG, Heuser JE, Donzell WC, et al. Caveolin, a protein component of caveolae membrane coats. *Cell* 1992;68:673–682. [PubMed: 1739974]
- Rubin LJ. Primary pulmonary hypertension. *N Engl J Med* 1997;336:111–117. [PubMed: 8988890]
- Runo JR, Loyd JE. Primary pulmonary hypertension. *Lancet* 2003;361:1533–1544. [PubMed: 12737878]
- Wunderlich C, Schmeisser A, Heerwagen C, et al. Chronic NOS inhibition prevents adverse lung remodeling and pulmonary arterial hypertension in caveolin-1 knockout mice. *Pulm Pharmacol Ther* 2008;21:507–515. [PubMed: 18226570]
- Zhao YD, Courtman DW, Deng Y, et al. Rescue of monocrotaline-induced pulmonary arterial hypertension using bone marrow-derived endothelial-like progenitor cells: efficacy of combined cell and eNOS gene therapy in established disease. *Circ Res* 2005;96:442–450. [PubMed: 15692087]
- Zhao YY, Liu Y, Stan RV, et al. Defects in caveolin-1 cause dilated cardiomyopathy and pulmonary hypertension in knockout mice. *Proc Natl Acad Sci U S A* 2002;99:11375–11380. [PubMed: 12177436]
- Zhao YY, Zhao YD, Mirza MK, et al. Persistent eNOS activation secondary to caveolin-1 deficiency induces pulmonary hypertension in mice and humans through PKG nitration. *J Clin Invest* 2009;119:2009–2018. [PubMed: 19487814]

Figure 1.

Model of eNOS activation-induced PH. Cav1 is a scaffolding protein of caveolae and acts as a critical negative regulator of eNOS. Under physiological conditions, low-output eNOS-derived NO maintains normal pulmonary vascular resistance and pulmonary arterial pressure. Cav1 may also interact with other molecule(s) through its scaffolding domain and regulate ROS production. Accordingly, Cav1 deficiency (*Cav1*^{-/-} mice or IPAH patients) results in persistent eNOS activation and formation of peroxynitrite in the pulmonary vasculature. The resultant increase of nitrate stress causes PKG tyrosine nitration and impairs its kinase activity. Impaired PKG signaling induces pulmonary vascular remodeling and vasoconstriction, and thereby pulmonary hypertension. PM, plasma membrane.