Pathogenesis of pulmonary hypertension: a case for caveolin-1 and cell membrane integrity

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Mathew R. Pathogenesis of pulmonary hypertension: a case for caveolin-1 and cell membrane integrity. Am J Physiol Heart Circ Physiol 306: H15–H25, 2014. First published October 25, 2013; doi:10.1152/ajpheart.00266.2013.—Pulmonary hypertension (PH) is a progressive disease with a high morbidity and mortality rate. Despite important advances in the field, the precise mechanisms leading to PH are not yet understood. Main features of PH are loss of vasodilatory response, the activation of proliferative and antiapoptotic pathways leading to pulmonary vascular remodeling and obstruction, elevated pressure and right ventricular hypertrophy, resulting in right ventricular failure and death. Experimental studies suggest that endothelial dysfunction may be the key underlying feature in PH. Caveolin-1, a major protein constituent of caveolae, interacts with several signaling molecules including the ones implicated in PH and modulates them. Disruption and progressive loss of endothelial caveolin-1 with reciprocal activation of proliferative pathways occur before the onset of PH, and the rescue of caveolin-1 inhibits proliferative pathways and attenuates PH. Extensive endothelial damage/loss occurs during the progression of the disease with subsequent enhanced expression of caveolin-1 in smooth muscle cells. This caveolin-1 in smooth muscle cells switches from being an antiproliferative factor to a pro-proliferative one and participates in cell proliferation and cell migration, possibly leading to irreversible PH. In contrast, the disruption of endothelial caveolin-1 is not observed in the hypoxia-induced PH, a reversible form of PH. However, proliferative pathways are activated in this model, indicating caveolin-1 dysfunction. Thus disruption or dysfunction of endothelial caveolin-1 leads to PH, and the status of caveolin-1 may determine the reversibility versus irreversibility of PH. This article reviews the role of caveolin-1 and cell membrane integrity in the pathogenesis and progression of PH.

caveolin-1; endothelial cells; pulmonary hypertension; smooth muscle cells

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In 1891, Ernst von Romberg, a German physician, described histological changes in pulmonary hypertension (PH) as pulmonary vascular sclerosis (30). Since then remarkable advances have been made in the understanding of PH, but the pathogenesis of PH still remains elusive, partly because a number of diseases can lead to PH and multiple signaling pathways are implicated in PH. Furthermore, not all signaling pathways are activated in a given patient, and their activation may depend on the stage of the disease. Experimental data suggest that the vascular changes occur before the onset of PH (47, 48). Therefore, it is not surprising that by the time the diagnosis of PH is made, most patients have significant pathological changes in pulmonary vasculature. Based on the underlying pathology, PH is categorized in 5 major groups (117). Group 1: the term pulmonary arterial hypertension (PAH) is assigned to this group, which includes idiopathic and heritable PAH and PAH associated with congenital heart diseases (CHDs), connective tissue diseases, portal hypertension, infection, drug toxicity, and persistent PH of the newborn. Pulmonary venoocclusive disease and pulmonary capillary hemangiomatosi are included as a subcategory. Although rare, PAH is a progressive disease with a high morbidity and mortality rate. The average survival time for the untreated patients is reported to be about 2.8 yr (23). Recent advances in therapy have improved the quality of life and delayed the progression of the disease but have not provided a cure. In a recent series of 354 patients with PAH on therapy, the 3-year survival rate was reported to be 58% (52). Furthermore, the patients on prostacyclin therapy for a longer duration exhibit worse pulmonary vascular pathology compared with the ones
on a shorter duration (103), underscoring the progressive nature of the disease despite modern therapy. Group 2: PH secondary to left heart diseases; group 3: PH secondary to chronic lung diseases and associated hypoxia; group 4: PH associated with chronic thrombo-embolism; group 5 includes PH associated with a variety of unrelated diseases such as sarcoidosis, metabolic and hematologic disorders, myeloproliferative diseases, chronic renal failure on dialysis and thyroid diseases. Irrespective of the underlying disease, endothelial dysfunction, enhanced vasoconstrictor reactivity, activation of proliferative and antiapoptotic pathways, vascular remodeling, elevated pulmonary artery pressure, and right ventricular hypertrophy are the main features of PH, eventually developing neointima, plexiform lesions, and right ventricular failure, leading to premature death. Several lines of evidence suggest that the endothelial dysfunction may be the key underlying defect in the pathogenesis of PH. Endothelial dysfunction results in an imbalance between vasorelaxation and vasoconstriction, cell proliferation and apoptosis, anti-inflammation and proinflammation, and antithrombotic and thrombogenic functions, leading to progressive vascular remodeling [reviewed in Mathew (82)].

Endothelial Cell Function

Endothelial cells (ECs) form a nonthrombogenic monolayer, a critical interface between circulating blood and underlying smooth muscle cells (SMCs), and act as a selective barrier to macromolecules and protect SMCs from elements of blood, direct pressure, and shear stress [reviewed in Mathew (82)]. ECs play an important role in inflammatory responses and participate in numerous metabolic functions. Under normal conditions, the apoptosis rate in ECs is very low, but the activated ECs exhibit a reduction in the EC surface layer, the glycocalyx, and an increased rate of apoptosis. Glycocalyx coats the luminal surface of ECs and forms an important barrier, modulates permeability, and prevents leukocyte and platelet adhesion to ECs. It mediates the shear-induced nitric oxide (NO) from ECs, and thus participates in mechanotransduction. Endothelial glycocalyx is shed in response to inflammation, oxidized LDL, abnormal blood shear stress, TNF-α, ischemia-perfusion injury, and redox stress. Vascular EC is one of the earliest sites of injury during inflammation, and the loss of glycocalyx increases intravascular leukocyte and platelet adhesion to ECs and activates inflammatory and coagulatory cascades (9, 37, 122). In response to infection and inflammatory mediators, ECs secrete increased amounts of interleukin (IL)-6, and upregulate intracellular adhesion molecule and vascular adhesion molecule, which spread over the surface of ECs. In addition to intracellular adhesion molecule and vascular adhesion molecule, P-selectin released from Weibel Palade bodies allows rapid rolling and adhesion of leukocytes on the EC surface. Interestingly, E-selectin maintains this process. Interaction of leukocyte platelet EC adhesion molecule-1 (PECAM-1) and EC PECAM-1 leads to transmigration of leukocytes through the inter-EC junction and possibly also through ECs (40) [reviewed in Mathew (82)]. Since inflammation induces the activation of ECs leading to endothelial dysfunction, it is not surprising that inflammation plays a significant role in the pathogenesis of PH. Patients with underlying inflammatory and autoimmune diseases such as scleroderma (75), polyneuropathy, organomegaly, endocrinopathy M-protein, skin abnormalities (POEMS) syndrome (71), acquired immunodeficiency syndrome (21), sarcoidosis (94), and schistosomiasis (66) have a propensity to develop PH. Furthermore, patients with PH exhibit perivascular infiltrates of inflammatory cells (126), regulated on activation, normal T cell expressed and secreted (RANTES) associated with CD45 + inflammatory cells (26), and increased circulating levels of proinflammatory cytokines such as IL-1, IL-6 (51), fractalkine (7), and monocyte chemoattractant protein-1, currently known as chemokine (C-C motif) ligand 2 (57).

Juxtaposition of ECs and SMCs facilitates cross talk, and ECs maintain SMCs in a quiescent state. ECs facilitate in maintaining a balance between vasoconstriction and vasodilatation, cell proliferation and apoptosis, and between pro- and anticoagulation factors. In addition, ECs provide barrier function and participate in immune function [reviewed in Mathew (82)]. Myoendothelial gap junction plays an important role in Ca 2+ exchange between ECs and SMCs. Interestingly, endothelial Ca 2+ traverses to SMCs and from SMC Ca 2+ and inositol 1,4,5-triphosphate components cross into ECs. Ca 2+ from SMCs causes an increase in Ca 2+ levels in ECs, resulting in enhanced endothelium-dependent relaxation (54). However, superoxide from SMCs inhibits the endothelial NO, modulates vascular relaxation, and maintains contraction under physiological pressure (12). The mammalian target of rapamycin (mTOR) controls cell growth, and is pivotal for vascular reactivity. Recent studies indicate that mTOR activation may be linked to SMC proliferation in PH (46a). Importantly, in cell culture studies, it has been shown that the growth factors mediate the activation of mTOR in SMCs. In contrast, mTOR activation in ECs is induced by flow but not by growth factors. Importantly, SMCs inhibit flow-mediated activation of mTOR in ECs, and ECs inhibit growth factor-mediated activation of mTOR in SMCs (8). Furthermore, SMCs alter the expression of the factors involved in coagulation and fibrinolysis (109). Thus the cross talk between ECs and SMCs is of fundamental importance in maintaining vascular health. The alterations in the EC/SMC cross talk during injury are likely to impact vascular homeostasis.

Caveolae and Caveolin-1

Caveolae, a subset of specialized lipid rafts (50–100 nm), first described by Palade and Yamada (98, 133) in the 1950s are found on plasma membranes of a variety of cells including ECs and SMCs. They form an important signaling platform that compartmentalizes and integrates a number of signaling molecules and allows cross talk between different signaling pathways. Caveolin-1, a major protein (~22 kDa) constituent of caveolae that maintains the shape of caveolae, was identified in 1992 (68, 110). ECs have the highest levels of caveolin-1 [reviewed in Frank et al. (31)]. Caveolin-1 functions through protein-protein interaction and regulates and stabilizes several proteins including Src family of kinases, G proteins (α-sub-units), G protein-coupled receptors, H-Ras, PKC, endothelial NO synthase (eNOS), integrins, and growth factor receptors such as vascular endothelial growth factor receptor (VEGF-R) 2, epidermal growth factor receptor in an inhibitory conformation (20) [reviewed in Okamoto et al. (95) and Patel et al. (100)]. Caveolin-1 exerts negative regulation of the target
proteins within caveolae, through the caveolin-1 scaffolding domain (CSD, residue 82–101) (22).

Caveolin-1 regulates vascular tone. For optimal activation, eNOS is targeted to caveolae. Caveolin-1 inhibits eNOS through its interaction, and heat shock protein 90 (HSP90) binds to eNOS in a Ca2+-calmodulin-dependent manner, thereby reducing the inhibitory influence of caveolin-1 and increasing eNOS activity (41). However, caveolin-1 is essential for proper eNOS activation. Major ion channels including the regulatory system that controls store-operated Ca2+ entry are organized in caveolae. Interestingly, caveolae are the preferred site for Ca2+ entry when the endoplasmic reticulum stores are depleted (55) [reviewed in Isshiki and Anderson (56)]. Further studies have shown that transient receptor potential channels 1 and 4 and 1,4,5-trisphosphate receptor type 3 interact with CSD and regulate Ca2+ entry into ECs, and the caveolin-1 deletion results in a loss of store depletion-activated Ca2+ entry (91, 121). Caveolin-1 deletion-induced impaired Ca2+ entry has been shown to affect the production of endothelium-derived hyperpolarizing factor adversely (113). In addition, increase in free cytosolic Ca2+ concentration levels initiate prostacyclin synthesis (58). Furthermore, caveolin-1 reduces vascular permeability and tissue edema in eNOS-mediated inflammatory responses (16). Caveolin-1 and eNOS have a dynamic interrelationship [Mathew et al. (79)], and caveolin-1 regulates EC function [reviewed in Minshall et al. (85)].

Caveolin-1, also known as a tumor suppressor factor, interacts with a number of proliferative pathways implicated in PH. Downregulation of caveolin-1 leads to cell proliferation. Observed cell hyperproliferation in caveolin-1−/− mice has been attributed to the mitogen-activated protein kinase (MAPK) signaling pathway. Interestingly, caveolin-1 is negatively regulated by the MAPK pathway (29, 34). Furthermore, the CSD interacts with platelet-derived growth factor receptor (PDGF-R) that colocalizes in caveolae and inhibits PDGF signaling and is able to transform PDGF-induced proliferative signals into death signals (102). Overexpression of caveolin-1 inhibits PDGF-induced vascular SMC proliferation, and PDGF stimulation upregulates caveolin-1 mRNA but facilitates caveolin-1 protein degradation via lysosomal pathway (102, 134).

Increased mRNA expressions of PDGF and PDGF-R (α and β) have been reported in pulmonary arteries from patients with PH (101). Increased expression of PDGF-Rβ and its tyrosine phosphorylation were also found in the monocrotaline (MCT) and hypoxia models of PH. Importantly, treatment with tyrosine kinase inhibitor reduced the expression of PDGF-Rβ and its phosphorylation and reversed PH (114). These results support a role for PDGF in the pathogenesis of PH. Activation of signal transducer and activator of signaling (STAT) 3 is required for PDGF-induced cell proliferation, and the inhibition of the PDGF receptor results in the suppression of cell proliferation via the inactivation of STAT3 signaling (45) [reviewed in Mathew (83)]. STAT3 activation [tyrosine phosphorylated (PY)-STAT3] is emerging as an important factor in vascular diseases including PH. Activation of PY-STAT3 has been reported in a number of experimental models such as the MCT- (78), hypoxia- [reviewed in Mathew (81)], and myocardial infarction-induced PH (59), and also in ECs obtained from patients with idiopathic PAH (74). The downstream effects of PY-STAT3 are mediated by cyclin D1 (cell cycle regulator), survivin and B-cell lymphoma-extra large (Bcl-xL) (antiapoptotic factors), all reported to be upregulated in PH. Inhibition of STAT3 reduces the expression of cyclin D1, survivin, and Bcl-xL (36, 60) and prevents neointima formation in the carotid artery injury model (116). Caveolin-1 acts as a suppressor of cytokine signaling and inhibits PY-STAT3 activation and modulates pro-inflammatory cytokines (61). In addition, caveolin-1 promotes cell cycle arrest via a p53/p21WAF1/CIP1-dependent mechanism and regulates apoptosis via suppressing survivin (35, 125).

Figure 1 summarizes the interrelationship of caveolin-1 with PDGF and PY-STAT3 in PH.

Bone morphogenic protein receptor (BMPR) II, a receptor member of the TGF-β superfamily, plays an important role in PH. BMPRII signaling protects ECs from apoptosis and is essential for BMP-mediated regulation of SMC growth and differentiation. BMPRII gene mutation is well recognized in patients with familial and heritable PAH. BMPRII gene mutation was also found in 6% of patients with PAH and associated CHD. Furthermore, the reduction in the expression of BMPRII has been reported in patients with secondary PAH, albeit less than what was observed in patients with idiopathic PAH without BMPRII mutation (5, 108, 123). In addition, a reduction in the expression of BMPRII has been reported in the MCT and hypoxia-induced PH (86, 107). Smurf-1, which induces ubiquitination and degradation of BMPRII, is upregulated in both MCT and hypoxia models of PH, thus contributing to the reduction in BMPRII expression (86). Interestingly, a part of BMPRII colocalizes with caveolin-1 in caveolae (105). BMP2 initiates Smad signaling by binding to its receptors in caveolae, leading to phosphorylation of BMPR1α. Caveolin-1 has been shown to directly interact with BMPRII in mouse aortic SMCs. This interaction is mediated by CSD and is regulated by caveolin-1 phosphorylation. Importantly, downregulation of caveolin-1 results in a loss of BMP-dependent Smad phosphorylation and gene regulation. The loss of caveolin-1 leads to a reduction in BMPRII membrane localization, resulting in de-
increased association of BMPRII with BMPR1a (13, 130). Furthermore, persistent activation of PY-STAT3 leads to a reduction in the BMPRII protein expression, and BMP2 inhibits PY-STAT3 and downregulates Bcl-xl, resulting in the induction of apoptosis (15, 64). Loss of BMPRII in vivo and in vitro has been shown to increase the production of cytokines such as IL-6, monocyte chemoattractant protein-1, and TGF-β (42). It is worth noting here that the reduction in endothelial caveolin-1 associated with increased IL-6 expression and PY-STAT3 occurs in human and experimental forms of PH (11, 49, 51), which in turn can influence BMPRII expression and function. From these studies one could surmise that caveolin-1 and BMPRII cooperate to maintain vascular health.

Effects of Injury to EC Membrane

Vascular ECs bear the major brunt of injury such as inflammation, chemical/drug toxicity, ventilation-induced injury, hypoxia, and shear stress that cause endothelial dysfunction. Resulting endothelial dysfunction leads to the loss of vascular relaxation mechanism coupled with the activation of proliferative and antiapoptotic pathways. Depending on the stimuli, ECs secrete several transducing molecules for participation in vascular tone and structure, inflammation, thrombosis, barrier function, cell proliferation, and apoptosis. Recent studies indicate that an injury can either disrupt EC membrane, resulting in the loss of endothelial caveolin-1, or lead to the perturbation of EC membrane without caveolin-1 loss but with caveolin-1 dysfunction, both leading to PH. In the former case, the progressive endothelial damage leading to EC loss is accompanied by enhanced expression of caveolin-1 in SMCs, which may negatively impact the course of the disease. In the latter case, SMCs do not acquire enhanced expression of caveolin-1, and, importantly, PH may be reversible [reviewed in Mathew (82)].

Disruption of EC Membrane

Loss of endothelial caveolin-1. Loss of endothelial caveolin-1 has been reported in several forms of experimental models of PH such as MCT- (78), myocardial infarction- (59), and VEGF-R blocker + hypoxia-induced PH (2). Importantly, reduction in the endothelial caveolin-1 protein and mRNA has been observed in patients with idiopathic PAH (2, 39). Austin et al. (6) recently reported caveolin-1 mutation associated with PAH. The expression of the endothelial caveolin-1 was reduced in these cases. In the MCT model, progressive loss of endothelial caveolin-1 and the activation of proliferative and antiapoptotic pathways occur before the onset of PH (48, 78). The rescue of caveolin-1 inhibits the proliferative pathways (PY-STAT3, cyclin D1 and D3) and attenuates PH (47, 60). Studies with caveolin-1 −/− mice have further underscored the importance of caveolin-1 in vascular health and disease. Caveolin-1 −/− mice are viable but with shortened life span (106). These mice exhibit hypercellular lung phenotypes, cardiomyopathy, systemic vasculopathy, and PH (27, 90, 136). In these mice, loss of caveolin-1 is associated with hyperactivation of eNOS, increased cGMP production, and uncoupling of eNOS, leading to the generation of reactive oxygen species (ROS) and nitrogen species. The eNOS-dependent generation of ROS is termed “uncoupling of eNOS,” which occurs under certain pathological state associated with relative deficiency in eNOS substrate. This oxidant and nitrosative injury is thought to be the underlying mechanism of PH in caveolin-1 −/− mice. Reexpression of endothelial caveolin-1 restores vascular and cardiac pathology and dysfunction. Interestingly, increased caveolin-1 has been shown to prevent eNOS-derived ROS by forming a complex with free eNOS (62, 90, 137). Furthermore, inhibition of eNOS by N3-nitro-L-arginine methyl ester prevents vascular remodeling and PH in caveolin-1 −/− mice. Interestingly, double knockout mice (caveolin-1 and eNOS) do not develop PH. The eNOS transgenic mice with increased endothelial eNOS expression, however, do not develop PH and are protected from hypoxia-induced PH (96, 132, 137). These studies indicate that caveolin-1 is essential not only for inhibiting proliferative pathways but also for maintaining physiological function of eNOS.

In the MCT model, eNOS expression is increased at 48 h and 1 wk post-MCT before the onset of PH, possibly as a compensatory mechanism. At this stage, eNOS remains functional as indicated by a relatively normal endothelium-dependent, NO-mediated relaxation response. At 2 wk post-MCT, PH is accompanied by a significant loss of endothelial caveolin-1 and a reduction in the expression of eNOS, although not significantly lower compared with the controls. It is, however, associated with a significant loss of eNOS-activating molecules HSP90 and Akt; low levels of NO, cGMP, and sulfhydryls; impaired endothelium-dependent vascular relaxation; and increased superoxide generation (47, 48, 76, 77). Altered HSP90 and eNOS interaction has been shown to uncouple eNOS activity and promote eNOS-dependent superoxide generation (104). This situation can be further aggravated by the loss of caveolin-1, because caveolin-1 can form a complex with free eNOS (62) and prevent superoxide formation. By 3 and 4 wk, with a further reduction in the eNOS expression, superoxide generation returns to normal (47).

Progressive loss of other endothelial membrane proteins such as Tie2, PECAM-1, and soluble guanylate cyclase occurring in parallel with the loss of caveolin-1 strongly suggests that there is a generalized disruption of EC membrane in the MCT model of PH. In addition, at 1 wk post-MCT before the development of PH, progressive activation of PY-STAT3 and increased expression of Bcl-xl occur (48, 78). At 2 wk post-MCT, there is evidence of PH and right ventricular hypertrophy; only 26 ± 3% of the arteries exhibit the presence of endothelial caveolin-1 compared with the controls with intact endothelial caveolin-1 in 100% of arteries (49). Immunofluorescence studies revealed the expression of PY-STAT3 and proliferating cell nuclear antigen in pulmonary arterial ECs with significant reduction in caveolin-1 expression (78), supporting the view that the loss of endothelial caveolin-1 is associated with the reciprocal activation of proliferative pathways. At 4 wk post-MCT, in addition to the progressive loss of endothelial caveolin-1 (15 ± 2% arteries with intact endothelial caveolin-1), ~9% of these arteries exhibit a loss of von Willebrand factor (vWF). Seventy percent of the arteries exhibiting vWF loss displayed enhanced expression of caveolin-1 in SMCs. All arteries expressing vWF loss had endothelial caveolin-1 loss, but all arteries expressing caveolin-1 loss did not have vWF loss. Importantly, at 2 wk post-MCT, there was no loss of vWF (49) or any evidence of enhanced expression of caveolin-1 in SMCs. Loss of vWF, indicative of progressive endothelial damage and extensive EC damage and/or loss, is followed by enhanced caveolin-1 expression in SMCs as...
shown in Fig. 2. vWF is formed in trans-Golgi network and stored in Weibel Palade bodies. As they mature, they become responsive to secretagogues such as thrombin and histamine. Weibel Palade bodies store a number of molecules that are required for hemostasis, inflammation, cellular proliferation, and angiogenesis. These include vWF, P-selectin, angiopoietin-2, endothelin-1, and endothelin-converting enzyme, IL-8, calcitonin gene-related peptide, and proteoglycan, a member of TNF-α receptor family readily available for their designated function (84). The loss of vWF is indicative of extensive EC damage and/or loss. This view is further supported by the observation that the increased circulating levels of vWF (65), angiopoietin-2 (67), and ECs (119) are associated with irreversible PAH. In their series of patients with PAH associated with CHD, Smajda et al. (119) did not observe any difference in the levels of circulating progenitor cells in the irreversible PAH, reversible PAH, or the controls.

Loss of ECs is thought to increase the proliferation of apoptosis-resistant cells and has been shown to release TGF-β1 (a promoter of SMC proliferation) and VEGF (inhibitor of apoptosis), promoting SMC proliferation (111, 112). Caveolin-1 plays an important role in TGF-β signaling. The internalization of the TGF-β receptors by caveolin-1-associated rafts results in the degradation of the receptors, but internalization by early endosome nonlipid rafts increases the signaling and increased TGF-β signaling inhibits caveolin-1 expression [reviewed in Del Galdo et al (24) and Zhang et al. (135)].

Enhanced expression of caveolin-1 in SMCs. Loss of endothelial caveolin-1 and enhanced expression of caveolin-1 in SMCs have been reported in patients with idiopathic PAH (99). Furthermore, SMCs isolated from pulmonary arteries of these patients exhibit not only enhanced expression of caveolin-1 but also high intracellular Ca\(^{2+}\) levels, increased capacitative Ca\(^{2+}\) entry, and DNA synthesis. Silencing caveolin-1 inhibits both capacitative Ca\(^{2+}\) entry and DNA synthesis (99). This clearly indicates that caveolin-1 in SMCs with its enhanced expression has become proproliferative. Similarly, in patients with chronic obstructive pulmonary disease and associated PH, endothelial caveolin-1 loss is accompanied by an enhanced expression of caveolin-1 in SMCs. Caveolin-1 alterations, however, were not observed in chronic obstructive pulmonary disease patients without associated PH (50). Surprisingly, loss of endothelial caveolin-1 in PAH associated with CHD has been observed early during childhood (25). Increased pulmonary blood flow and shear stress in the presence of elevated pulmonary artery pressure disrupts endothelial caveolin-1 and PECAM-1 with subsequent loss of vWF, leading to enhanced expression of caveolin-1 in SMCs. However, increased pulmonary blood flow in the presence of normal pulmonary artery pressure does not disrupt cell membrane. Endothelial caveolin-1, PECAM-1, and vWF remain well preserved, and SMCs do not exhibit enhanced expression of caveolin-1 (25). Enhanced expression of caveolin-1 in SMCs, accompanying extensive EC damage or loss in a child with drug-induced PAH, has been reported to lead to neointima formation and a loss of response to PH therapy (80). The disruption and loss of ECs may be a prerequisite for enhanced expression of caveolin-1 in SMCs, which may ultimately lead to neointima formation.

In the MCT model of PH, enhanced expression of caveolin-1 in SMCs is accompanied by a significant increase in the expression and the activity of matrix metalloproteinase (MMP) 2 (49). SMCs from patients with idiopathic PAH also exhibit increased MMP2 activity (70), which may facilitate extracellular matrix degradation, cell migration, and eventual neointima formation. MMP2 belongs to a gelatinase subgroup of a family of zinc-containing proteolytic enzymes considered to be responsible for the breakdown of extracellular matrix. The activation of MMP2 increases SMC proliferation and migration. Importantly, membrane type 1-MMP, a physiological activator of pro-MMP2, is preferentially localized in caveolae, and caveolin-1 negatively regulates membrane type 1-MMP and MMP2 activity and inhibits cell migration (4, 19, 92). The increased expression and activity of MMP2 in the presence of enhanced expression of caveolin-1 in SMCs suggest that caveolin-1 has lost its inhibitory function.

Under normal conditions, caveolin-1 is reported to participate in the regulation of Ca\(^{2+}\) entry in SMCs enabling vasoconstriction (33) [reviewed in Ishihi and Anderson (56)]. Disruption of caveolin-1 has been shown to reduce myogenic tone and impair contractile responses to several agonists (3, 28). In addition, caveolin-1 keeps proliferative pathways including extracellular signal-regulated kinases inactive in caveolae. Both in vivo and in vitro, the arterial SMCs lacking caveolin-1 display proliferation abnormalities, migration, and altered Ca\(^{2+}\) handling (43). In a carotid artery injury model, caveolin-1 \(^{−/−}\) mice develop neointimal lesions associated with the upregulation of the MAPK pathway and cdc42 (44). In response to cyclic stretch, caveolin-1 in SMCs (in vitro) translocate from caveolae to a noncaveolar site within the plasma membrane. Furthermore, the translocated caveolin-1 mediates extracellular signal-regulated kinase activation and facilitates cell cycle progression and cell proliferation. Stretch-
induced cell proliferation is abolished by caveolin-1 antisense treatment, indicating the pivotal role of caveolin-1 in stretch-induced cell proliferation. During cyclic stretch, caveolin-1 is critically involved in proliferative signaling, and the mechanically triggered stimuli may participate in the cell proliferation and progression of vascular proliferative disease. Importantly, caveolin-1−/− SMCs do not proliferate on exposure to cyclic stretch (63, 115). The extensive damage and/or loss of ECs may impose wall strain and stretch induced by elevated pressure directly onto SMCs resulting in translocation of caveolin-1 from caveolae, which may participate in the progression of PH. The enhanced expression of caveolin-1 in SMCs in PH not only appears to lose its inhibitory capacity but actively facilitates cell proliferation and cell migration, subsequently leading to neointima formation (80, 99). Caveolin-1 may in part be responsible for SMC phenotype change from contractile to synthetic. It is worthy of note that caveolae are more numerous in contractile phenotype, and caveolin-1 mainly localizes in caveolae (124). The dual role of caveolin-1 in PH is not unlike what is observed in cancer. In early stages of cancer, caveolin-1 acts as a tumor suppressor, but in late stages it promotes metastasis and multidrug resistance, thus negatively influencing the prognosis [reviewed in Mathew (81)]. The effect of caveolin-1 in PH may well be cell specific and dependent on its conformation and the stage of the disease.

Perturbation of EC Membrane

Postcapillary PH and PH associated with emphysema and interstitial lung diseases often do not lead to EC proliferation and neointima formation. In a large series of patients with PH, plexiform lesions were found to be uncommon in arteries with an average thickness < 20% of the internal diameter. Plexiform lesion is usually localized near the bifurcation of the arteries. It consists of elastic fragmentation and plexus of small vessels within an artery. However, arteries with dilatation lesions that show dilated segments with degenerative changes in SMCs did not develop plexiform lesions (127–129). Furthermore, the hypoxia-induced PH is reversible upon removal of hypoxic condition (17, 118). Unlike the MCT model that is associated with a progressive loss of caveolin-1 and subsequent reduction in the expression of eNOS, there is no loss of endothelial caveolin-1 or eNOS expression in the hypoxia model of PH (87). During hypoxia, caveolin-1 forms a tight complex with eNOS, resulting in the dysfunction of both molecules, as evidenced by low bioavailability of NO and the activation of PY-STAT3 and PDGF signaling pathways (122a) [reviewed in Mathew (81) and Murata et al. (88)]. Interestingly, the disruption of the eNOS/caveolin-1 complex with statin treatment improves functions of both molecules, resulting in the reversal of PH (89). In hypoxia-induced PH, vasoconstriction may have an important role. Hypoxia impairs agonist-induced Ca2+ entry in ECs, leading to impaired endothelium-dependent, NO-mediated relaxation response. Impaired Ca2+ handling in caveolin-1−/− mice can be restored by genetic reconstitution of caveolin-1 in ECs. Caveolin-1 regulates transient receptor potential channel-1 localization via the interaction of CSD peptide (87, 91, 121). Recent studies have shown that cholesterol depletion blunts Ca2+ entry in ECs from controls, but not in ECs from chronic hypoxia-exposed rats. Chronic hypoxia leads to a reduction in the membrane cholesterol and impairs agonist-induced Ca2+ entry. The introduction of cell permeable CSD restores Ca2+ entry in these cells (97). These observations indicate that caveolin-1 may be required for Ca2+ entry in ECs for vasoactivity. Since caveolin-1 and eNOS coupling occurs at the CSD level, it is likely that because of the tight coupling between eNOS and caveolin-1 during hypoxia, enough CSD may not be available for the agonist-induced Ca2+ entry and the exogenous CSD can restore the Ca2+ entry.

In premature infants, the disruption of normal pulmonary vascular and bronchial development results in reduced cross-sectional area of vasculature, leading to increased pulmonary vascular resistance and PH. Interestingly, pulmonary arteries from infants with respiratory distress syndrome/bronchopulmonary dysplasia do not exhibit any breach in the intimal layer. Despite elevated pulmonary artery pressure, there is no loss of endothelial caveolin-1, PECAM-1, vWF, or enhanced expression of caveolin-1 in SMCs. However, in infants who had associated inflammatory disease significant enough to cause EC damage and endothelial caveolin-1 disruption resulted in the loss of vWF and enhanced expression of caveolin-1 in SMCs (25). The most likely explanation for this is that the perturbation of EC membrane without physical disruption, despite associated caveolin-1 dysfunction, does not expose SMCs to direct pressure and shear stress, which may prevent SMCs from alterations in caveolin-1 expression. This may be the reason why the reversal of PH and vascular remodeling are possible in these cases. In contrast, associated inflammation in infants with respiratory distress syndrome/bronchopulmonary dysplasia destroys the EC membrane integrity and disrupts endothelial caveolin-1. The progressive EC damage results in EC loss with subsequent enhanced expression of caveolin-1 in SMCs (25) which may participate in further cell proliferation, cell migration, and facilitate in the progression of PH toward irreversibility.

Burke et al. (17) recently showed that sustained hypoxia creates a pulmonary artery-specific proinflammatory microenvironment with the accumulation of inflammatory cells, adhesion molecules, and cytokines. Return to normoxia results in the resolution of proinflammatory microenvironment and attenuation of PH. It is very likely that the inflammatory cytokines and adhesion molecules in long-term and persistent hypoxic conditions will eventually cause EC injury, leading to EC disruption and loss. Loss of ECs is likely to lead to enhanced expression of caveolin-1 in SMCs with further cell proliferation and cell migration and progress toward irreversibility. The proposed model of hypoxia-induced PH is shown in Fig. 3.

Caveolin-1 and Plexiform Lesions

Dysregulated angiogenesis in PH leads to plexiform lesions in small pulmonary arteries. Angiogenesis is a process of new vessels sprouting from existing vessels. VEGF and its receptors play a key role during embryonic vasculogenesis and also during normal and pathological angiogenesis. Increased expression of VEGF and its receptor VEGF-R2 has been reported in plexiform lesions in the lungs of patients with idiopathic PAH and PAH secondary to CHD (38, 46, 127). Interestingly, similar to human plexiform lesions, increased expression of VEGF and VEGF-R2 has been reported in the complex lesions found in the VEGF-R2 blocker + hypoxia model of severe PH.
In the case of increased pulmonary blood flow, which is likely to lead to enhanced expression of caveolin-1 in SMCs and subsequent deregulated angiogenesis. It is significant that pneumonectomy itself has no effect on the pulmonary vasculature. This is not unlike CHD with increased pulmonary blood flow and normal pulmonary artery pressure, without any disruption of EC membrane or caveolin-1, or enhanced expression of caveolin-1 in SMCs. In contrast, CHD associated with increased pulmonary blood flow, elevated pulmonary artery pressure, and shear stress results in the loss of endothelial caveolin-1, vWF, and increased expression of caveolin-1 in SMCs (25). If the underlying cardiac defects in these patients are not corrected in a timely fashion, PH is likely to progress to the irreversible form. These observations further support the view that EC loss is essential for further cell proliferation, neointima formation, and deregulated angiogenesis leading to plexiform lesions.

Although there is a reduction in eNOS expression in small arterioles from hypertensive lungs, the plexiform lesions in PH exhibit strong expression of eNOS (73). Similarly, Berger et al. (10) have reported increased expression of eNOS and inducible NO synthase in plexiform lesions in PAH associated with CHD. Zhao et al. (137) using Western blot analysis showed reduced caveolin-1 expression in the lung tissue from patients with idiopathic PAH associated with normal eNOS expression and increased PKG-1 nitration. They further showed that the inhibition of eNOS or treatment with superoxide dismutase mimic significantly attenuated PH in caveolin-1−/− mice. Thus eNOS-mediated generation of superoxide has a significant role in the progression of PH. Caveolin-1, which regulates eNOS-derived NO as well as superoxide and is involved in the sequestration of uncoupled eNOS, prevents eNOS oxidase activity and inhibits superoxide formation (62). In addition, caveolin-1-dependent inhibition of eNOS-superoxide generation is enhanced with phosphorylation of eNOS, especially Thr495 phosphorylation (18). These observations indicate that the reduction in caveolin-1 expression with increased or normal expression of eNOS in ECs in plexiform lesion is likely to make the disease worse by increasing oxidant and nitrosative stress. Oxidant stress is an important feature in the lungs of patients with idiopathic PAH (14). Based on these studies, one could argue that the addition of antioxidants as an adjuvant to PH therapy may be necessary to protect the vasculature from ongoing oxidant injury.

In summary, caveolae and caveolin-1 play an important role in maintaining vascular health. In the MCT model of PH and some forms of human PAH, endothelial disruption and loss of membrane proteins including endothelial caveolin-1 and reciprocal activation of proliferative pathways have been reported. EC disruption is progressive, resulting in the loss of multiple proteins and eventually leading to EC loss, exposing SMCs to direct pressure and shear stress. This leads to enhanced expression of caveolin-1 in SMCs and possibly translocation of caveolin-1 from caveolae to a non-caveolar site. Caveolin-1 at this stage becomes a proproliferative factor and actively participates in cell proliferation, cell migration, and neointima formation. Increased expression of eNOS and VEGF coupled with a reduced expression of caveolin-1 in these neointimal ECs may deregulate angiogenesis and facilitate the formation of irreversible PH.
of plexiform lesions. In addition, ECs in plexiform lesions have reduced expression of caveolin-1 but with normal or increased levels of eNOS expression leading to uncoupling of eNOS and superoxide generation, exerting negative influence and leading to irreversible PH. In contrast to the MCT model, the perturbation of EC membrane observed in the hypoxia model of PH is not associated with the disruption of EC membrane or the loss of endothelial caveolin-1 or enhanced expression of caveolin-1 in SMCs. Caveolin-1 forms a tight complex with eNOS, resulting in dysfunction of both molecules. Since there is no breach in the endothelial layer, the underlying SMCs are not exposed to direct pressure or shear stress, and these SMCs do not exhibit enhanced expression of caveolin-1. Removal of the hypoxia or disruption of caveolin-1/eNOS complex with statins results in the reversal of PH and vascular remodeling. However, if there is persistent inflammation or added shear stress in the presence of PH, resulting EC disruption may lead toward the path of irreversible form, suggesting that the endothelial membrane integrity and caveolin-1 function may determine the reversibility versus irreversibility of PH. An ideal approach to PH therapy may be to rescue EC membrane integrity, which at present does not seem feasible. The alternate approach may be to modulate caveolin-1 expression.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
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