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Caveolin-1 Deficiency Signaling Novel Mechanisms of Pulmonary Hypertension

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Abstract

Pulmonary hypertension is an unremitting disease characterized by progressive increase of pulmonary vascular resistance and vascular remodeling. Due to poor understanding of the molecular basis of the pathogenesis, there are currently limited options available for the treatment of this devastating disease. Recent studies with *Cav1$^{-/-}$* mice and other genetically modified animal models as well as experimental animal models of pulmonary hypertension have demonstrated the critical role of Caveolin-1 deficiency in the pathogenesis of pulmonary hypertension. Here, we will review the current knowledge about the role of Caveolin-1 signaling in the mechanisms of pulmonary hypertension focusing on protein kinase G nitration and STAT3 activation and provide insights into the molecular basis of the pathogenesis of human pulmonary hypertension.

Introduction

Pulmonary hypertension (PH) is characterized by a progressive increase in pulmonary vascular resistance and vascular remodeling [1-2]. PH features several clinical manifestations such as vasoconstriction, pulmonary vessel remodeling, intravascular thrombosis, and endothelial dysfunction [1-4]. The stiffening of artery walls as well as lumen narrowing make it more difficult for the heart to pump blood through the pulmonary circulation. Such strain on the heart causes the right ventricle to eventually weaken and fail. Progressive PH is currently an incurable disease with a high mortality rate, due to poor understanding of the molecular mechanisms underlying the pathogenesis and hence limited options available for the prevention and treatment of progressive PH [5-6]. Idiopathic pulmonary arterial hypertension (IPAH) is the most severe form of PH, which without treatment leads to right heart failure and premature death [2-4].

Caveolae are 50 to 100-nm vesicular invaginations of the cell plasma membrane [7]. Besides their critical role in mediating vesicular trafficking, caveolae serve as signal transduction microdomains to concentrate and orchestrate many signaling events [8-9]. Caveolin-1 (Cav1), a 22 kDa protein abundantly expressed in many non-muscle cell types, especially in endothelial cells and adipocytes, is the scaffolding protein of caveolae [10-11]. Cav1 binds many signaling molecules such as eNOS, receptor and non-receptor tyrosine kinase receptors, G protein-coupled receptors, GTPase, calcium channels, integrins, as well as components of the mitogen-activated protein kinases, and regulates their functions [12-18]. The integral nature of Cav1 in signaling transduction implicates its important roles in health and diseases. Given the comprehensive review by Mathew R. regarding the cell-specific dual role of Cav1 in the pathogenesis of PH [19], we here discuss the recent findings about the pathogenic roles of defective Cav1 signaling in the mechanisms of PH and focus on Cav 1 deficiency-induced nitration of ptotien kinase G (PKG) as well as STAT3 activation.

Genetic deletion of Cav1 induces PH in mice

To study the (patho) physiological role of Cav1, several groups have generated *Cav1$^{-/-}$* mice a decade ago. Surprisingly, *Cav1$^{-/-}$* mice are viable although there is lack of caveolae in Cav1-expressing cells such as endothelial cells [20-22]. However, it has been shown that *Cav1$^{-/-}$* mice develop PH (greater than 80% increase of pulmonary arterial pressure) [22]. These mice exhibit right ventricle hypertrophy associated with marked increase of right ventricular contractility and diastolic function [22] and increased pulmonary vascular resistance as well as pulmonary vascular remodeling evident by increased medial thickness and muscularization of distal pulmonary vessels [23]. Studies have also shown decreased pulmonary artery density and defective pulmonary artery filling [24]. Consistent with the role of Cav1 deficiency in promoting cell proliferation in vitro [25-27], *Cav1$^{-/-}$* lungs exhibit hypercellularity and alveolar septal thickening [20-21, 23]. Endothelial re-expression of Cav1 in *Cav1$^{-/-}$* background rescues the pulmonary hypertensive phenotypes seen in *Cav1$^{-/-}$* mice [28]. Taken together, these data provide unequivocal evidence about the critical role of Cav1 deficiency in the pathogenesis of PH.

Cav1 deficiency is involved in the pathogenesis of experimental models of PH

Rats treated with one dose of monocrotaline (MCT) show loss of Cav1 prior to the onset of severe PH [29]. The progressive loss of Cav1 in rat lungs starts as early as 48h and peaks at two weeks post-MCT challenge. Evidence of Cav1 involvement in PH is also observed in other animal models of PH such as myocardial infarction [30] and SU5416/hypoxia-induced severe PH [31]. In the latter studies, Cav1 expression is selectively decreased in the complex cellular arterial lesions [31]. Importantly, administration of a cell-permeable Cav1 mimetic peptide corresponding to the putative scaffolding domain (amino acids 82-101) prevents loss of Cav1 and development of PH in MCT-challenged rats

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[32]. These studies further demonstrate the pathogenic role of Cav1 deficiency in the development of PH. However, it remains unclear how treatment with the Cav1 mimetic peptide prevents loss of Cav1. Previous studies have shown that this peptide can inhibit eNOS activity and reduces inflammation in vivo [33].

Persistent eNOS activation and resultant PKG nitration secondary to Cav1 deficiency plays a causal role in the pathogenesis of PH in $Cav1^{-/-}$ mice

Cav1 is a critical negative regulator of eNOS activity. We and others have shown chronic eNOS activation and marked increase of nitric oxide (NO) production in the circulation and also in lung tissues in $Cav1^{-/-}$ mice [20-23]. To gain insight into the role of eNOS activation in the mechanism of PH, we have used a double knockout (DKO) mouse model with genetic deletions of both Cav1 and NOS3 (encoding eNOS) [23]. In contrast to $Cav1^{-/-}$ mice, DKO mice do not develop pulmonary hypertension exhibiting normal right ventricular systolic pressure and pulmonary vascular resistance. Pulmonary vascular remodeling is also inhibited in DKO lungs. Consistently, treatment of $Cav1^{-/-}$ mice with L-NAME, a pan-NOS inhibitor reverses the hypertensive pulmonary phenotypes seen in $Cav1^{-/-}$ mice [23,34]. These data demonstrate the causal role of eNOS activation in the pathogenesis of PH in $Cav1^{-/-}$ mice. eNOS activation secondary to Cav1 deficiency leads to formation of the damaging reactive oxygen species (ROS) peroxynitrite which modifies proteins and regulates their function through tyrosine nitration [23]. Prominent nitrotyrosine immunostaining is evident in $Cav1^{-/-}$ pulmonary vasculature. PKG, the downstream target of NO signaling responsible for vasorelaxation formation, is found nitrated and its kinase activity is impaired in $Cav1^{-/-}$ lungs. In vitro, direct incubation of PKG-1α with peroxynitrite impairs its kinase activity. Specifically, mutation of tyrosine residues 345 and 549 in the kinase domain of human PKG-1α abolishes PKG nitration-mediated impairment of PKG activity. In the DKO lungs, PKG nitration is inhibited and PKG kinase activity is normalized. L-NAME treatment and superoxide scavenging also inhibit PKG nitration and reverse PH in $Cav1^{-/-}$ mice. Restoration of PKG activity by overexpression of PKG-1 reduces right ventricular systolic pressure and pulmonary vascular resistance. These data suggest that oxidative/nitrative stress-induced PH in $Cav1^{-/-}$ mice is ascribed to impaired PKG kinase activity through tyrosine nitration. It is interesting to determine whether treatment with the Cav1 mimetic peptide in $Cav1^{-/-}$ mice will result in decreased PKG nitration through inhibition of eNOS activation and thereby reverses PH.

However, eNOS-derived NO is in general considered to be protective [35]. $eNOS^{-/-}$ male mice under normoxic condition are mildly pulmonary hypertensive (less than 20% increase in RVSP) [36,37]. In response to mild hypoxia, both male and female eNOS deficient mice develop severe PH [38]. Contrary to our finding where increased eNOS activity in $Cav1^{-/-}$ mice is shown to be crucial for the mechanism of PH, in previous studies the overproduction of NO in eNOS transgenic mice inhibits the increase in RVSP and lung vascular remodeling as well as right ventricular hypertrophy induced by chronic hypoxia [39]. Our recent study demonstrates increased superoxide production in $Cav1^{-/-}$ lungs (Zhao et al., unpublished observations), which reacts with eNOS-derived NO to form peroxynitrite and thereby induces PH in $Cav1^{-/-}$ mice. In agreement with our finding, NO alone did not induce tissue injury even at high concentration [40], whereas NO in the presence of superoxide produces peroxynitrite and subsequently causes severe nitrative stress and tissue injury [41-43]. Importantly, inhibition of NO production by L-NAME reverses the pulmonary alterations responsible for PH in $Cav1^{-/-}$ mice [23,34]. Together, these data show a critical role of Cav1 in the pathogenesis of PH through its

tight regulation of eNOS activity such that disruption of the negative regulation of eNOS by Cav 1 leads to pulmonary vascular remodeling and PH through PKG nitration.

PKG nitration-mediated impairment of PKG activity may be a common mechanism of PH induced by tissue hypoxia and inflammation

PKG nitration and resultant impairment of PKG activity in the pulmonary vasculature is also involved in the mechanism of PH induced by hypoxia [44,45]. It has been shown that hypoxia impairs PKG activity and PKG-mediated relaxation in ovine fetal intrapulmonary veins through both downregulation of PKG expression and tyrosine nitration of PKG [45]. Other studies also show decreased PKG activity and resultant attenuated vasodilatory responses to exogenous NO and cGMP in rats following chronic hypoxia [44]. The impaired PKG activity is not due to decreased PKG expression as PKG expression is in fact upregulated in the rat pulmonary vasculature following four week hypoxia [44]. Although there is conflict about the expression levels of PKG in these two reports, both point to the role of PKG nitration in impairing its kinase activity. It is likely that decreased PKG activity in rats following chronic hypoxia is the result of hypoxia-induced PKG nitration. Recently, Lisanti and colleagues have shown that stromal cells lacking Cav1 mimic a constitutive hypoxic phenotype [46]. These Cav1 null cells may experience mitochondria dysfunction, leading to nitrative stress as evident by peroxynitrite formation. In contrast to the observation in $Cav1^{-/-}$ mice, hypoxia-induced PKG nitration is eNOS-independent but iNOS-dependent [45]. Thus, it is possible that both tissue hypoxia and inflammation will result in increased nitrative stress and PKG nitration through iNOS. In turn, PKG nitration impairs PKG activity and thereby induces PH.

STAT3 activation secondary to Cav1 deficiency is attributed to the pathogenesis of PH

STAT3, a member of the signal transducer and activator of transcription is translocated to nuclei upon phosphorylation and subsequently induces expression of genes involved in cell proliferation and anti-apoptosis including Bcl-xL, cyclin D1 and survivin. All of these molecules have been implicated in PH [47,48]. Cav1 deficiency results in STAT3 phosphorylation in $Cav1^{-/-}$ lungs [30]. In MCT-treated rat lungs, decreased Cav1 expression is associated with increased phosphorylation of STAT3 and upregulation of cyclin D1 [30,32]. Administration of Cav1 mimetic peptide restores Cav1 expression and inhibits STAT3 phosphorylation and upregulation of cyclin D1, which is accompanied by inhibition of PH [32]. Given that STAT3 phosphorylation is a downstream effector of proinflammatory cytokine interleukin-6, and hypoxia activates STAT3, STAT3 phosphorylation may be an important mediator of PH associated with inflammation in human and experimental forms of PH [49].

Cav1 deficiency is implicated in the pathogenesis of PH in humans

Plexiform lesion samples taken from deceased patients with severe PH show abnormal growth of endothelial cells as well as smooth muscle cells. Cells of both types in these lesions have dramatically decreased levels of Cav1, whereas tissue from other parts of the lung has ubiquitous expression. Heme oxygenase 1 levels also mirrored Cav1, where lack of antioxidant properties may exacerbate oxidative stress and PH, as discussed above. The authors suggest the lack of Cav1 as a mechanism for hyperproliferation and apoptosis-resistance of these cells [31]. Other studies of human subjects suffering from IPAH showed decreased levels of Cav1 in whole lung lysates [50].

Vessels from these patients show almost a complete lack of Cav1 in the endothelium. However, Cav1 expression was overexpressed in pulmonary vascular smooth muscle cells. Employing lung samples from IPAH patients, we have observed decreased Cav1 expression in IPAH lung tissues [23]. eNOS activity and PKG nitration are drastically increased in IPAH lung tissues in the absence of marked changes of eNOS and PKG expression [23].

Paradoxically, impaired bioavailability of NO is a key underlying feature for clinical and experimental pulmonary hypertension. It is possible that NO reaction with greater amount of reactive oxygen species in lung tissues from IPAH patients results in decreased bioavailability of NO although eNOS is activated. In addition, eNOS is robustly expressed in the plexiform lesions of IPAH lungs [51]. On the basis of the findings from *Cav1$^{-/-}$* mouse lung, it is likely that Cav1 deficiency in endothelial cells in IPAH pulmonary vasculatures results in eNOS activation and PKG nitration and thereby induces PH in IPAH patients.

Conclusions

PH is characterized by progressive increases in pulmonary vascular resistance (PVR) and vascular remodeling, which without treatment leads to right heart failure and death. Prominent oxidative/nitrative stress is a hallmark of the pathology of severe PH [52-53]. Tissue hypoxia, ischemia, and inflammation all contribute to ROS production in the lung tissue of patients with severe PH [54-55]. Recent studies from genetically modified mouse models, experimental animal models of PH and lung samples from patients with severe PH including IPAH have demonstrated the critical role of Cav1 deficiency in the pathogenesis of PH. Cav1 deficiency induces nitrative stress through eNOS activation and ROS production, which causes PKG nitration and resultant impairment of PKG activity. Impaired PKG activity induces vasoconstriction and vascular remodeling and thereby PH (Figure 1). These studies provide a novel insight into the molecular basis of severe PH associated with oxidative/nitrative stress. It would be of great value to identify the source of superoxide which is essential for formation of peroxynitrite and PKG nitration and the underlying signaling
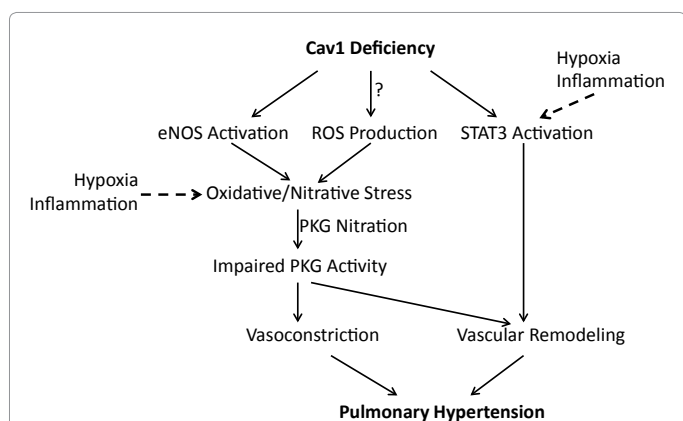
pathway(s) activated by Cav1 deficiency. Thus, targeting such signaling pathway(s) to inhibit superoxide production and resultant PKG nitration may represent a novel therapeutic strategy for the prevention and treatment of severe PH including IPAH. Other studies also show Cav1 deficiency leads to

activation of STAT3. Activated STAT3 induces cell proliferation and apoptosis resistance and thereby contributes to vascular remodeling (Figure 1). Thus, targeting PKG nitration may represent novel therapeutic strategies for the prevention and treatment of PH.

Acknowledgements

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Figure 1: Cav1 deficiency induces pulmonary hypertension. In *Cav1$^{-/-}$* mice, Cav1 deficiency induces eNOS activation and ROS production to form peroxynitrite which modifies PKG through tyrosine nitration. PKG nitration impairs PKG activity and thereby induces pulmonary vasoconstriction and vascular remodeling leading to development of pulmonary hypertension. Increased eNOS activity and PKG nitration concomitant with decreased Cav1expression are observed in lung tissues from IPAH patients. Tissue hypoxia and inflammation may augment oxidative/nitrative stress and thereby PKG nitration. In addition, Cav1 deficiency activates STAT3. Tissue hypoxia and inflammation also results in STAT3 activation. Activated STAT3 induces vascular remodeling and thereby contributes to the pathogenesis of PH.

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