Pulmonary arterial hypertension (PAH, or group 1 pulmonary hypertension) is a progressive, insidious, and fatal illness of the pulmonary microvasculature. Proliferative remodeling of the small resistance arteries results in an elevation of mean pulmonary arterial pressure (mPAP) and total pulmonary vascular resistance (PVR), increasing the workload on the right ventricle (RV) until it eventually fails. Although these hemodynamic parameters are useful for clinical diagnosis, alone they have limited prognostic value; furthermore, vasodilator therapy aimed at reduction of PVR has achieved only modest success, and increases in patient survival are attributed primarily to earlier diagnosis and intervention. These limitations have motivated research focused on identifying measurable physiological parameters that could both predict mortality and serve as measures for therapeutic efficacy.

Decreased pulmonary arteriolar compliance (PAC) is a major factor contributing to the increased RV workload and failure in PAH. PAC measures a vessel’s ability to deform under loading, and as a blood vessel stiffens its compliance decreases. Total vessel compliance is estimated as stroke volume divided by pulse pressure (PP). This estimation alone is a strong predictor of survival in idiopathic and familial PAH. Although proximal artery stiffness has received a great deal of attention in hypoxic pulmonary hypertension and is important for vascular remodeling changes PAC, especially in the distal microvasculature and modulated by vessel diameter, and PAC, a more complete representation of pulmonary hemodynamics, and inflammation. Abnormalities in cell–cell and cell–ECM force transduction also contribute to decreased PAC, and these alterations are driven primarily by abnormal integrin expression and disrupted cytoskeletal regulation. In this brief review, we first address the effects of distal PAC on PAH progression, and how changes in distal vascular stiffness contribute to increased RV workload and failure. We next turn our attention to the cellular and molecular pathways that link the causes of decreased PAC, we pay special attention to small-molecule mediators of ECM regulation, mechanotransduction, and intercellular force transduction because many of these mediators represent potential therapeutic targets.

Influence of Vessel Stiffness Changes on RV Failure in PAH

RV overload and failure are the ultimate cause of death in PAH. Classically, RV failure is attributed to the RV’s inability to adapt to an increased workload caused by elevated PVR. However, PVR alone provides limited prognostic value. Moreover, vasodilators—intended to decrease PVR by widening the vessel lumen and restoring flow rates—provide only transitory relief with minimal effect on mortality. Because PVR is a measure for the intrinsic resistance to steady state flow, measurements of PVR inherently fail to capture the oscillatory pumping action of the RV. Oscillatory work accounts for >25% of the RV workload fraction under normal and diseased conditions, significantly more than in systemic circulation. A more complete representation of pulmonary hemodynamics takes into account both PVR, primarily localized to the microvasculature and modulated by vessel diameter, and PAC, an intrinsic mechanical property of the vessel wall and distributed throughout the entire vasculature. Furthermore, because PAC is a critical determinant of RV oscillatory work, a greater understanding of how PAC is decreased in PAH will assist the development of therapies designed to target the underlying causes of RV overload.

In systemic hypertension, evidence suggests that increased arterial stiffness may precede elevated blood pressures in...
some instances and is well correlated with disease severity. Similarly, both the stiffness of the large conduit pulmonary arteries and the overall compliance of the entire vascular bed predict mortality in patients with PAH. Normally, the high compliance of conduit vessels dissipates pulse wave energy and decreases PP and RV afterload, a phenomenon captured by measures of pulmonary vascular impedance. Research using hypoxic pulmonary hypertension animal models illustrates that decreased proximal PAC contributes significantly to the RV workload by elevating PP. Stiffening of the large arteries also increases pulse reflections in the vasculature, further augmenting total PP.

Although the increased stiffness of the conduit arteries undoubtedly contributes to disease progression and is comparatively well studied, proximal large artery compliance comprises only 15% to 25% of the total PAC, with the remainder distributed across the entirety of the arterial bed. Compliance of the distal vasculature is important in normal physiology for regulating pulmonary flow rates, especially during exercise. Direct measurements of distal artery compliance are normally difficult to obtain because of the small size and limited accessibility of the vessels. Efforts to develop methods from a combination of pressure–diameter curves, echocardiography, and nonlinear regression analysis have yielded reasonable estimates. Models explaining the pressure–flow relationship in the pulmonary circulation as a function of PAC (rather than PVR) also provide more complete representations of pulmonary hemodynamics, accurately predicting how changes in blood viscosity alter the pressure–flow relationship. Studies correlating PVR with total PAC have also shown that the product of the 2 values remains constant during the early stages of PAH therapy although it is possible that certain drugs may alter this relationship in the diseased state.

This inverse hyperbolic association suggests that small initial changes in PVR result in large changes in vessel compliance and consequently PP and RV oscillatory workload, and these initial large changes in PAC are evident early in the disease.

The distal and proximal arteries communicate with one another in a cycle of positive feedback that detrimentally influences disease progression, elevating vessel stiffness through a combination of increased ECM production and rearrangement and augmented intercellular force transmission (Figure 1). Chronic vasoconstriction and vessel remodeling cause decreased PAC in the distal vasculature, which may be important in mediating pathological changes early in the disease state, including compromising the endothelial barrier function and further enhancing ECM synthesis. Proximal artery walls thicken in response to elevated mPAP and also stiffen, accumulating collagen, fragmenting elastin, and increasing impedance and RV workload. Proximal arterial stiffening also amplifies pulse wave velocity and wave reflections. These reflections transmit increased pressures back to the proximal arteries from the distal circulation, increasing the PP. Amplified pulse wave transmission to the distal vasculature, also a result of proximal arterial stiffening, increases shear stress on the endothelium and results in an inflammatory response that drives further distal vascular remodeling, vasoconstriction, ECM production, and subsequent loss of distal PAC, further elevating mPAP and also stiffen. Understanding the mechanisms that drive alterations in distal arterial compliance may allow for new drug targets capable of preventing disease progression in the early stages of development and help break the cycle of positive feedback that partially makes PAH so intractable to traditional therapies.

**Cellular and Molecular Mechanisms Responsible for Modulating Arterial Stiffness in PAH**

Proliferative remodeling of the small arteries is driven by multiple interconnected pathways and involves changes in the ECM and the vascular cell population. Traditionally, increased vascular stiffness has been attributed to alterations in ECM content, especially collagen accumulation. However, recent evidence suggests that altered ECM organization, dysregulation of cell–ECM and cell–cell force transmission, and intrinsic stiffening of PASMCs may also decrease PAC. The molecular pathways that drive changes during PAH development include the downstream effects of mutations in the TGF-β superfamily of receptors (most notably BMPR2), a chronic inflammatory state, and abnormal serotonin signaling, whereas dysregulated integrin signaling and cytoskeletal abnormalities contribute to cell-level changes in vessel wall stiffness.

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**Figure 1.** Changes in distal vessel pulmonary arteriolar compliance (PAC) begin a positive feedback cycle advancing the progression of pulmonary arterial hypertension and ultimately leading to increased right ventricular (RV) oscillatory workload and failure. mPAP indicates mean pulmonary arterial pressure; PAEC, pulmonary arterial endothelial cell; and PP, pulse pressure.
ECM Content and Remodeling

The content and arrangement of the ECM are critical regulators of blood vessel mechanics. Collagen is actively synthesized in the small muscular arteries of patients with idiopathic PAH and may be an important contributor to the loss of small artery compliance. Radioactively labeled collagen is actively synthesized by PASMCs of the media and the adventitia of small and large arteries in PAH, increasing resistance to strain at both early and late deformations. Regional accumulations of medial and adventitial collagen also colocalize with decreases in PAC in sickle cell--associated PAH. PASMCs in the media and fibroblasts in the vessel adventitia are generally identified as the cellular source for this regional collagen accumulation although pulmonary arterial endothelial cells that transdifferentiate into PASMC-like cells likely also contribute. Proper alignment of collagen is an important determinate of its mechanical properties. In systemic arteries, collagen fibers are aligned both longitudinally and circumferentially, and this organization provides resistance to lateral and radial strain, respectively. Collagen fiber organization is regulated in mature vessels by the balanced activity of matrix metalloproteinase (MMP) and tissue inhibitors of MMPs. Ample evidence from several animal models of PAH suggests that both MMPs and tissue inhibitors of MMPs are abnormally regulated during disease; however, the roles of the specific subtypes differ substantially depending on the animal model used, making it difficult to determine their respective contributions from animal models alone. In cultured PASMCs isolated from patients with idiopathic PAH, both MMP-2 and MMP-9 expression and activity are elevated, and both circulating MMP-9 and type III collagen degradation products correlate well with PAH disease severity. Elevated MMP-2, MMP-3, and MT1-MMP expression in the endothelial plexiform lesions of patients with PAH also colocalizes with areas of collagen IV degradation; however, whereas alterations in the expression of these MMPs can cause global changes in artery stiffness in systemic circulation, to date there are no studies correlating their expression with colocalized alterations in pulmonary vessel wall mechanics. Basement membrane degradation by MMP-2 also promotes production of tenascin-C, a glycoprotein that amplifies the response of PASMCs to various growth factors. MMP-induced PASMC proliferation and collagen production provides a possible explanation for how simultaneously increased collagen breakdown and synthesis can result in net increased collagen deposition. This deposition, coupled with disordered alignment, likely contributes to decreased PAC.

Where collagen imparts rigidity to the vessel wall, elastin imparts compliance. Mice with variable vascular elastin expression exhibit gradually increasing mPAP that inversely correlates with the amount of elastin expressed. However, other studies have demonstrated concomitant increases in elastin and collagen content and arterial stiffness. Serine elastase is responsible for elastin degradation and is upregulated in PAH, colocalizing with pathological neointimal lesions in the small arteries. Elastin degradation likely contributes to increased vessel stiffness because it causes a transfer of load bearing to the stiffer collagen fibers, especially at lower pressures. Similar to MMPs, serine elastase induces growth factor release (such as fibroblast growth factor) and tenascin-c production; therefore, serine elastase inhibition is expected to exert antiproliferative and proapoptotic effects on PASMCs and reverse remodeling of distal arteries. It is likely that elastin degradation decreases PAC through a combination of these effects although the magnitude of each effect on overall PAC remains unclear.

Altered ECM content and remodeling in PAH are driven primarily by alterations in the TGF-β/BMP signaling axis, chronic inflammation in the vessel adventitia, and disrupted serotonin signaling (Figure 2). Mice with a deletion of BMPR1a from their PASMCs develop elevated mPAP with exposure to prolonged hypoxia and show evidence of increased adventitial collagen deposition and elastin lamina disruption in areas of BMPR1a deletion. However, although ECM changes induced by the BMPR1a deletion decrease proximal PAC, it is ultimately protective against distal vascular remodeling. TGF-β1 signaling through Alk1 is an important mediator of collagen deposition, and BMP2 mutations cause increased TGF-β1 production and an abnormal proliferative response to TGF-β1. TGF-β1 can also activate and is in turn activated by MMP-9, suggesting a feed-forward signaling mechanism that advances vascular remodeling. Release of inflammatory cytokines (such as interleukin-6 and interleukin-1β) by macrophages in the vessel adventitia also contributes to the initial activation of MMPs, and the activation of these macrophages is a direct consequence of BMPR2 loss of function.

Abnormal serotonin signaling is also an important contributor to both collagen production and elastin degradation in PAH. Plasma serotonin levels are increased in patients with PAH, and pulmonary arterial endothelial cells in the distal microvasculature are identified as one source for this elevation. Abnormal serotonin signaling is also an important contributor to both collagen production and elastin degradation in PAH. Plasma serotonin levels are increased in patients with PAH, and pulmonary arterial endothelial cells in the distal microvasculature are identified as one source for this elevation. In systemic sclerosis, a disease often associated with PAH, serotonin induces fibroblast collagen synthesis by TGF-β1-dependent signaling through the 5-hydroxytryptamine2B receptor. 5-Hydroxytryptamine2B is upregulated in the small arteries of patients with idiopathic PAH, and vascular remodeling after hypoxia is absent in 5-hydroxytryptamine2B-deficient mice. In addition to regulating collagen production, serotonin can be used to cross-link matrix proteins by the enzyme transglutaminase-2 through a process known as serotonylation, further increasing vascular stiffness. Transglutaminase-2 is regulated by nitric oxide (NO), a vasodilator produced by pulmonary arterial endothelial cells and known to be significantly downregulated in PAH. Serotonin signaling also regulates elastase activity in PAH by inducing expression of the calcium-binding protein S100A4. S100A4 is overexpressed in the PASMCs of the remodeled small muscular arteries in pediatric patients with PAH, and mice overexpressing S100A4 develop PAH and small-vessel remodeling. In addition to promoting PASMC proliferation, S100A4 induces production of serine elastase.
by PASMCs, contributing to elastic lamina disruption and decreasing PAC. Although the mechanism of this induction is poorly defined, S100A4 expression is dependent partly on serotonin signaling through the 5-hydroxytryptamine1B receptor and activity of the serotonin transporter SERT.

**Cell–ECM Force Transmission and Intrinsic Cell Stiffness**

Transmission of force between cells of the vascular walls is also an important mediator of vessel stiffness. Force transmission between cells and their environment is accomplished functionally through integrins, a class of matrix-binding proteins. The cytoplasmic domains of integrins are associated focal adhesions (FAs), large protein complexes that directly transmit forces between the ECM and the cytoskeleton. These forces are also translated by FAs into chemical signaling pathways in a process known as mechanotransduction. Integrin binding and FA organization help to mediate the stiffening of systemic arteries, and impaired regulation of FA organization contributes to age-related aortic stiffening. Although the contributions of integrin force transmission and mechanotransduction to the development of systemic hypertension are increasingly well studied, relatively little is known about how integrins influence PAC in PAH. Integrin expression is significantly altered in PASMCs localized to the small pulmonary arteries of monocrotaline and hypoxia-treated rats. Although altered ECM deposition patterns may regulate some integrin subunits, nonspecific regulation of other subunits (specifically α5, β1, and β3) seems to contribute to disease progression by advancing vascular remodeling and PASMC contractility directly.

Elevated activity of the protein kinase Src is characteristic of idiopathic and hereditary PAH and could provide a link between integrin dysregulation and decreased PAC. Src regulates cell–ECM force transmission by controlling levels of FA kinase, and ultimately its integrin-cytoskeletal attachments, at the cell surface. When Src signaling is elevated, FA kinase levels at the cell surface increase and lead to greater transmission of force between the cell and ECM. Src activity is also regulated itself by integrin engagement and FA kinase activation: inhibition of FA kinase prevents PAH in monocrotaline-treated rats by decreasing Src activity and reversing the migratory phenotype of PASMCs. Elevated Src activity is characteristic of PAH; markers of Src activity are elevated in idiopathic PAH, and mutations in the BMPR2 receptor can cause a loss of Src functional inhibition leading to elevated...
signaling. Reduction of Src activity partially explains the beneficial effects of tyrosine kinase inhibitors in PAH therapy. Although this benefit has traditionally been associated with reductions in cell proliferation, it is also possible that reducing Src activity could alter abnormal patterns of integrin engagement and signaling and thereby decrease vascular stiffness.

Prolonged integrin engagement leads to permanent rearrangements in cytoskeletal organization that could contribute to a long-term decrease in PAC. Cell–ECM force transmission largely depends on integrin attachment to the noncontractile cortical cytoskeleton underlying the cell membrane. Chronic vasconstriction of smooth muscle cells, as occurs in PAH, causes a reorganization of the noncontractile cytoskeleton favoring net polymerization. This response facilitates low-energy maintenance of decreased vessel diameter and disengagement of the contractile apparatus. This process is also integrin dependent: as the vascular smooth muscle cell contracts, integrin contacts with the ECM increase concurrently with the stiffness of the cortical cytoskeleton, and cell stiffening does not occur in the absence of integrin-cytoskeletal attachments. Initially, these changes begin as adaptive by enabling the cell to withstand increased force transmission, but dysregulation of the underlying molecular mediators could pathologically alter cytoskeletal organization and consequently induce maladaptive changes in cell stiffness.

Changes in the actin cortical cytoskeleton organization—as seen in continuously contractile smooth muscle cells—have the potential to alter PAC significantly by inducing an intrinsic stiffening of PASMCs independent of transient cell contraction. Small-molecule mediators of actin polymerization are known to be abnormally regulated in PAH and as a consequence of the BMPR2 mutation (Figure 2). Upregulation of Rac1 in BMPR2 mutant mice causes a decrease in actin fiber stability and induces actin reorganization and polymerization. In addition, a large body of work demonstrates Rho kinase activity (the downstream effector of RhoA and mediator of F-actin polymerization) is increased in PAH, and inhibition of Rho kinase alleviates PAH development although the extent of this improvement is limited. LIMK1 phosphorylates cofilin, preventing cofilin from inhibiting actin polymerization. The cytoplasmic tail domain of BMPR2 directly inhibits LIMK1 activity, and mutations in this domain lead to increased LIMK1 activity and consequently increased actin polymerization. LIMK1 is also regulated by the same Rho GTPases that are dysregulated in hereditary PAH, suggesting that BMPR2 mutations contribute to cytoskeletal disruption via multiple mechanisms. However, additional studies are needed to clarify whether restoration of cortical cytoskeleton dynamics could appreciably restore compliance to the diseased pulmonary vessel wall.

Conclusions and Future Directions

Decreased PAC contributes substantially to an elevated RV workload in PAH. Increased stiffness of the proximal pulmonary arteries confers elevated impedance to flow and elevated PPs and has been studied extensively in the context of hypoxic pulmonary hypertension. Although proximal arterial stiffness is important in PAH, vascular remodeling and consequential stiffening of the smaller branch arteries and muscular arteries may also provide important contributions to disease development. In this review, we have briefly covered the emerging mechanistic studies linking initial genetic changes to alterations in PAC and how these mechanisms advance disease progression. In general, the aspects of vascular remodeling responsible for increasing vessel stiffness and decreasing PAC include changes in the content and organization of ECM, increased transmission of force between the ECM and vascular cells, and an intrinsic stiffening of the cells themselves driven by changes in cytoskeletal organization. Many of these changes stimulate feed-forward mechanisms whereby alterations in distal vessels further drive stiffening of the proximal pulmonary arteries and vise versa. New medications for PAH on the horizon could serve to alleviate some of these changes: Rho Kinase inhibitors, serotonin receptor antagonists, tryptophan hydroxylase inhibitors, Src inhibitors, interleukin-6 antibodies, and drugs targeting the BMP2/TGF-β1 pathway are all proposed therapeutics that may address the remodeling pathways discussed here. However, there are currently no approved drugs and few under active clinical investigation designed to target mechanisms responsible for decreased PAC directly. Many unanswered questions remain, and a shift in research focused on the molecular drivers underlying PAC changes is required to identify future therapeutic targets.

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