CHEST

Phenotypic Diversity of Vascular Smooth Muscle Cells in Pulmonary Arterial Hypertension

Implications for Therapy

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Pulmonary arterial hypertension (PAH) is a progressive incurable condition that is characterized by extensive remodeling of the pulmonary circulation, leading to severe right-sided heart failure and death. Similar to other vascular contractile cells, pulmonary arterial smooth muscle cells play central roles in physiological and pathologic vascular remodeling because of their remarkable ability to dynamically modulate their phenotype to ensure contractile and synthetic functions. The dysfunction and molecular mechanisms underlying their contribution to the various pulmonary vascular lesions associated with PAH have been a major focus of research. The aim of this review is to describe the medial and nonmedial origins of contractile cells in the pulmonary vascular wall and present evidence of how they contribute to the onset and progression of PAH. We also highlight specific potential target molecules and discuss future directions that are being explored to widen the therapeutic options for the treatment of PAH.

CHEST 2022; 161(1):219-231

KEY WORDS: contractile/synthetic phenotype; pulmonary hypertension; pulmonary vascular remodeling; smooth muscle heterogeneity; translational targets

Precapillary pulmonary hypertension (PH) is hemodynamically defined by a resting mean pulmonary arterial pressure > 20 mm Hg, together with pulmonary vascular resistance ≥ 3 Wood units in the presence of a normal left-sided heart filling pressure (≤ 15 mm Hg).[1] The term pulmonary arterial hypertension (PAH) encompasses a heterogeneous group of incurable pulmonary cardiovascular disorders that share a similar clinical presentation, hemodynamic characteristics, and therapeutic management.

ABBREVIATIONS: BMPRII = bone morphogenetic protein receptor type II; Ca^{2+} = calcium; CXCL12 = C-X-C motif chemokine ligand-12; CXCR = CXC receptor; EC = endothelial cell; ECM = extracellular matrix; EndoMT = endothelial-mesenchymal transition; GDF = growth differentiation factor; iPAH = idiopathic PAH; K^{+} = potassium; OPN = osteopontin; PA-SMC = pulmonary artery smooth muscle cell; PAH = pulmonary arterial hypertension; PDGF = platelet-derived growth factor; PDGFR = platelet-derived growth factor receptor; PDK = pyruvate dehydrogenase kinase; PH = pulmonary hypertension; RTK = receptor tyrosine kinase; SASP = senescence-associated secretory phenotype; SMC = smooth muscle cell; TGF-β = transforming growth factor beta; VSMC = vascular smooth muscle cell

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FUNDING/SUPPORT: B. Lechartier is a recipient of a research grant from the Fondation Juchum and the Ligue Pulmonaire Vaudoise. N. Berrebeh is a recipient of a PhD fellowship from the Ile de France region (ARDoc Health).

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DOI: https://doi.org/10.1016/j.chest.2021.08.040

PAH is diagnosed when the aforementioned hemodynamic criteria are met and once other causes of precapillary PH (eg, chronic thromboembolic PH [group 4], PH because of chronic lung disease [group 3]) are ruled out.[1] Current PAH therapy via approved drugs has been developed to favor pulmonary vasodilatation by targeting one of the three well-characterized pathways, namely the nitric oxide, endothelin-1, and prostacyclin signaling pathways.[2] Although current treatment options have markedly improved overall quality of life, exercise capacity, and long-term outcomes in PAH, 5-year survival remains low (~60%).[3] Lung transplantation is the final therapeutic option if medications fail. Deciphering the molecular mechanisms involved in the progressive narrowing and thickening of blood vessels in PAH is therefore fundamental for the development of new therapeutic strategies that could be used in conjunction with existing agents.

Histologic lung lesions from patients with PAH are classically characterized by intense and progressive pulmonary vascular remodeling, exemplified by the accumulation of various resident vascular cell types within the pulmonary vessel wall (ie, pulmonary artery smooth muscle cells [PA-SMCs], endothelial cells [ECs], adventitial fibroblasts, pericytes), perivascular inflammatory cell infiltration, and endothelial dysfunction.[4] Such extensive vascular remodeling predominantly affects the small to midsize pulmonary arteries < 500 μm in diameter. Typically, neomuscularization and thickening of the media of these pulmonary vessels are observed. These vascular lesions are uniformly distributed throughout both lungs of patients with PAH and are accompanied by varying degrees of intimal thickening and perivascular inflammatory infiltrates. In addition, a more-or-less pronounced loss of precapillary arteries and pulmonary venous remodeling can also be observed, with the formation of neointimal lesions and other complex pulmonary arterial lesions (ie, plexiform and singular millimetric fibrovascular lesions).[3,4] Although the exact pathophysiology of PAH is still unknown, advances provide a better understanding of the three driving components of vascular remodeling: pulmonary endothelial dysfunction, vascular smooth muscle abnormalities, and dysregulation of the inflammatory and immune systems.[4,5]

We review here the current understanding of the molecular mechanisms by which vascular contractile cells (ie, PA-SMCs, adventitial fibroblasts, pericytes, other circulating and resident progenitors) accumulate and contribute to the onset and progression of PAH. We also discuss the potential therapeutic targets that could be explored to widen therapeutic options for the treatment of PAH.

PA-SMC Function and Dysfunction

Heterogeneity of Vascular Smooth Muscle Cell Phenotypes and Functions

Developmentally, vascular smooth muscle cells (VSMCs) originate from two main sources, namely the mesoderm and the neuroderm, which represents the ectoderm of the neural crest. Most smooth muscle cells (SMCs) within the vascular tree are of mesodermal origin and are formed mainly from mesenchyme, a tissue found mostly during embryogenesis, or from mesothelium via the epithelial to mesenchymal transition.[6] Such disparity in their embryonic origin, combined with their remarkable capacity to change their phenotype in response to local microenvironmental cues (ie, growth factors and inflammatory mediators, physical and mechanical forces, cell-cell and cell-matrix interactions), represent major challenges in the field, particularly for their rigorous identification and phenotypic characterization. Detailed examination of these major building blocks of the tunica media in proximal and distal bovine pulmonary arteries reveals significant differences in cellular heterogeneity between the pulmonary trunk and distal resistance vessels. Indeed, the bovine proximal pulmonary arterial media contain at least four different PA-SMC subpopulations, with distinctive marker profiles and morphologic traits, whereas the bovine distal pulmonary arterial media are composed of a uniform population of well-differentiated PA-SMCs with low proliferative capacity.[7,8] Such diversity of VSMCs along the proximal-distal axis in the pulmonary vascular bed may partially explain certain local specific arterial responses to various stimuli or pathologic conditions, as observed in the hypoxic response.

In the pulmonary circulation, the heterogeneity and phenotypic plasticity of the distinct subpopulations of VSMCs play essential roles, not only in the maintenance of vascular functions over time, but also in their adaptation to chronic changes in the local microenvironment. Indeed, each individual VSMC can reversibly go from a quiescent to a contractile or synthetic phenotype at any time and therefore acquire or lose proliferative and migratory potential and the capacity to synthesize the extracellular matrix (ECM) or

certain marker proteins (Fig 1). Under physiological conditions in adulthood, most PA-SMCs are quiescent or fated to maintain a contractile function, which is considered to be their differentiated state.[6,9] They are uniformly spindle-shaped or elongated, with blunt-ended or cigar-shaped nuclei. Various growth factors and inflammatory mediators (eg, platelet-derived growth factor [PDGF], transforming growth factor beta [TGF-β], retinoids, angiotensin-II, activins), together with changes in mechanical stimuli, epigenetics, and the composition and organization of the ECM, are among the major determinants of their transition from the contractile to synthetic type. During this shift, VSMCs acquire proliferative and migratory capacities and express specific protein markers.

A myriad of SMC-specific genes can be used as markers of the differentiation and maturation state. The most widely described are α-smooth muscle actin, smooth muscle-myosin heavy chain (also known as MYH11), and smoothelin, but SM22α (TAGLN2), smooth muscle-calponin, desmin, and meta-vinculin can also be used to indicate a mature contractile SMC phenotype.[10] Most of these proteins act as components of the contractile machinery and as regulators of contraction. During the switch to the dedifferentiated/synthetic phenotype, the expression of most of these contractile markers is downregulated, with a particular reduction in α-smooth muscle actin and smooth muscle-myosin heavy chain expression and the loss of smoothelin expression. Other markers are acquired during the transition to the synthetic phenotype (eg, SMemb/non-muscle myosin heavy chain isoform-B, cellular retinol binding protein-1, PDGF-A, I-caldesmon, collagen type I, connexin-43, osteopontin [OPN], syndecan-1/-4).[10] The plasticity of VSMCs can also be regulated by various specific microRNAs, including miR-133 and miR-125a-5b.[11]

Understanding the diversity of the SMC phenotypes in the vessel wall and their determinants is the subject of intense research and will help in the design of future therapeutic approaches.

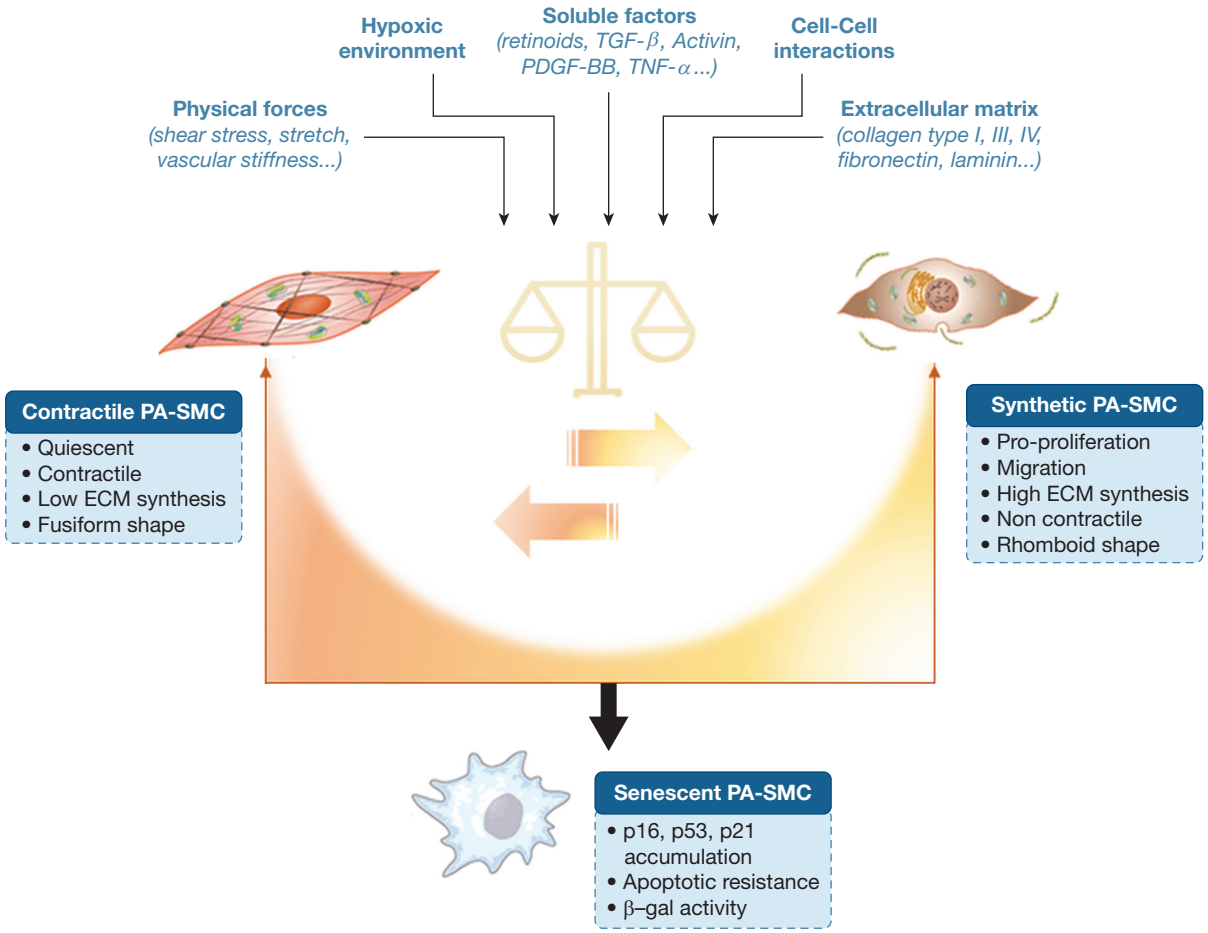

Figure 1 – *Phenotypic plasticity of PA-SMCs. ECM = extracellular matrix; PA-SMC = pulmonary arterial smooth muscle cell; PDGF-BB = platelet-derived growth factor-BB; TGF-β = transforming growth factor beta; TNF-α = tumor necrosis factor alpha.*

Changes in the Functional Properties of PA-SMCs in PAH

Inherent Intrinsic Abnormalities in PAH PA-SMCs: In PAH, dynamic and maladapted pulmonary vascular remodeling is accompanied by the acquisition and maintenance of a modified pro-proliferative apoptosis-resistant phenotype in the various contractile vascular cells in the arterial wall, including PA-SMCs, adventitial fibroblasts, and cells expressing myofibroblast-like characteristics.[4,12,13] Although these pathogenic processes are partially driven by qualitative and quantitative changes in various matrix and soluble factors within the pulmonary arterial wall, accumulating evidence also supports the notion that these various cell types may also exhibit several inherent intrinsic abnormalities. Consistent with this notion, PA-SMCs from patients with idiopathic PAH (iPAH) grow two to three times faster and for a longer period of time than control cells, indicating a higher proliferative capacity. Importantly, these characteristics are maintained even outside their pathologic microenvironment.[13,14] The known or suspected intrinsic abnormalities present in the various contractile vascular cell types in PAH include the dysfunction and/or loss of bone morphogenetic protein receptor type II (BMPRII) signaling, the dysregulation of potassium (K^+) channel homeostasis, and a reduction in signaling through the peroxisome proliferator-activated receptor-γ and forkhead box O-1 pathways, and constitutive activation of the nuclear factor-kappa B and prolyl hydroxylase 2/hypoxia inducible factor-1α axes.[4]

Heterozygous loss-of-function mutations in *BMPR2* (encoding BMPRII) or other PAH-related genes (ie, *ACVRL1* [encoding ALK1], *ENG* [encoding endoglin], *GDF2* [encoding BMP9], *BMP10* [encoding BMP10], *KCNK3* [encoding an acid-sensitive 2-pore domain K^+ channel, also known as TASK-1]) have been associated with several pathogenic features of PAH, including PA-SMC depolarization, proliferation, resistance to apoptosis, and constriction.[4,15] In PAH, the loss of BMPRII signaling in PA-SMCs and adventitial fibroblasts has also been associated with alterations of DNA damage response pathways, resulting in the progressive accumulation of unrepaired DNA lesions.[4] Although the mechanisms are still unclear, pulmonary vascular cells derived from patients who do not carry a specific mutation also exhibit downregulation of BMPRII signaling, highlighting the importance of the regulation of the BMP-growth differentiation factor (GDF) branch in PAH. Consequently, the lack of coordination between the TGF-β/activin/nodal branch and the BMP/GDF branch is currently viewed as the major molecular defect in PAH.[16,17]

These phenotypic changes are also closely linked with ion-channel remodeling, as reflected by the loss of calcium (Ca^{2+}) and K^+ homeostasis and Ca^{2+} sensitivity and the activation of several transcription factors. There is convincing evidence showing that heightened RhoA/Rho-kinase signaling substantially contributes to Ca^{2+} sensitization, muscle-cell contraction, proliferation, and vascular inflammation in PAH. Therefore, animal studies have shown that inhibition of RhoA/Rho-kinase attenuates Ca^{2+} sensitization, pulmonary pressure, and vascular remodeling in experimental models. It is well established that inhibition of K^+ channels (downregulation or blockade) indeed leads to plasma membrane depolarization, voltage-gated Ca^{2+} channel activation, and increased intracellular Ca^{2+} concentrations, resulting in PA-SMC constriction and proliferation. Interestingly, a small percentage of familial PAH cases has been indeed associated with genetic defects in *ABCC8* and *KCNK3*.[4,15]

A heightened response to several growth factor-stimulated signaling pathways is also observed in the various vascular contractile cell types in PAH. These growth factors include PDGF, epidermal growth factor, fibroblast growth factor-2, and nerve growth factor.[4,13,18-20] Although our understanding of the underlying molecular mechanisms is still limited, these abnormal responses have been partially ascribed to overexpression of the various receptor tyrosine kinases (RTKs) responsible for the activation of signal transduction and to alterations in the expression of proteins involved in the integration or amplification of the intracellular signal. It has been indeed reported that adaptor protein $p130^{cas}$, which represents a nodal signaling platform that conveys integrin and RTK signaling, is overexpressed in iPAH.[13]

Therefore, a better understanding of how these inherent intrinsic abnormalities alter vascular integrity and the balance between the various regulatory molecules that control the balance between vasoconstriction and relaxation and cell proliferation and apoptosis or senescence within the pulmonary artery wall is needed.

Energy and Metabolic Changes: Vascular contractile cells from patients with PAH are also able to adapt their metabolic status to satisfy changing bioenergetic and biosynthetic demands. Metabolic reprogramming

accompanying the switch to the dedifferentiated/synthetic phenotype has been documented in experimental and human PAH.[4] Numerous studies have indeed highlighted a switch from mitochondrial oxidative phosphorylation to glycolysis and dysregulation of several other major metabolic pathways, including those for amino acid metabolism and lipid oxidation.[21] Given the indispensability of cellular energy and metabolites for cell survival and growth, it is not surprising that during the process of vascular remodeling in PH, there are several indications that PA-SMCs, adventitial fibroblasts, and other contractile cells acquire a metabolically active phenotype over time. Such metabolic adaptation has been confirmed in experimental and human PAH by 18-fluorodeoxyglucose PET imaging.[22,23] It has also been demonstrated that mice lacking malonyl-CoA decarboxylase, a key enzyme involved in the regulation of fatty acid oxidation, exhibit attenuated acute hypoxic pulmonary vasoconstriction and are less susceptible to the remodeling of pulmonary vessels than wild-type mice.[24] The same authors also demonstrated that restitution of oxidative metabolism via the inhibition of pyruvate dehydrogenase kinase (PDK), an inhibitor of mitochondrial enzyme pyruvate dehydrogenase (the gatekeeping enzyme of glucose oxidation), and inhibition of fatty acid oxidation with trimetazidine can reverse the pulmonary vascular remodeling induced by chronic hypoxia in mice, and in monocrotaline-treated rats.[24] As already reviewed,[4,25,26] PA-SMCs from patients with idiopathic PAH also exhibit other dysfunctional metabolic pathways, including an increased hypoxia inducible factor signaling, an activation of fatty acid oxidation, and an enhanced glutamine metabolism (Fig 2). However, a more complete understanding of the metabolic reprogramming and perturbations and dysfunction that affect mitochondria and their dynamics in PAH is essential for the identification of a tolerable and effective metabolic therapy that does not counterregulate right ventricular maladaptation in patients with PAH. Further studies aiming to elucidate the effects of mechanical forces/shear stress and persistent inflammation on PAH-specific metabolic reprogramming are also necessary to strengthen our understanding of how these changes in metabolic status affect the diversity of the SMC phenotype and contribute to the onset and progression of PAH.

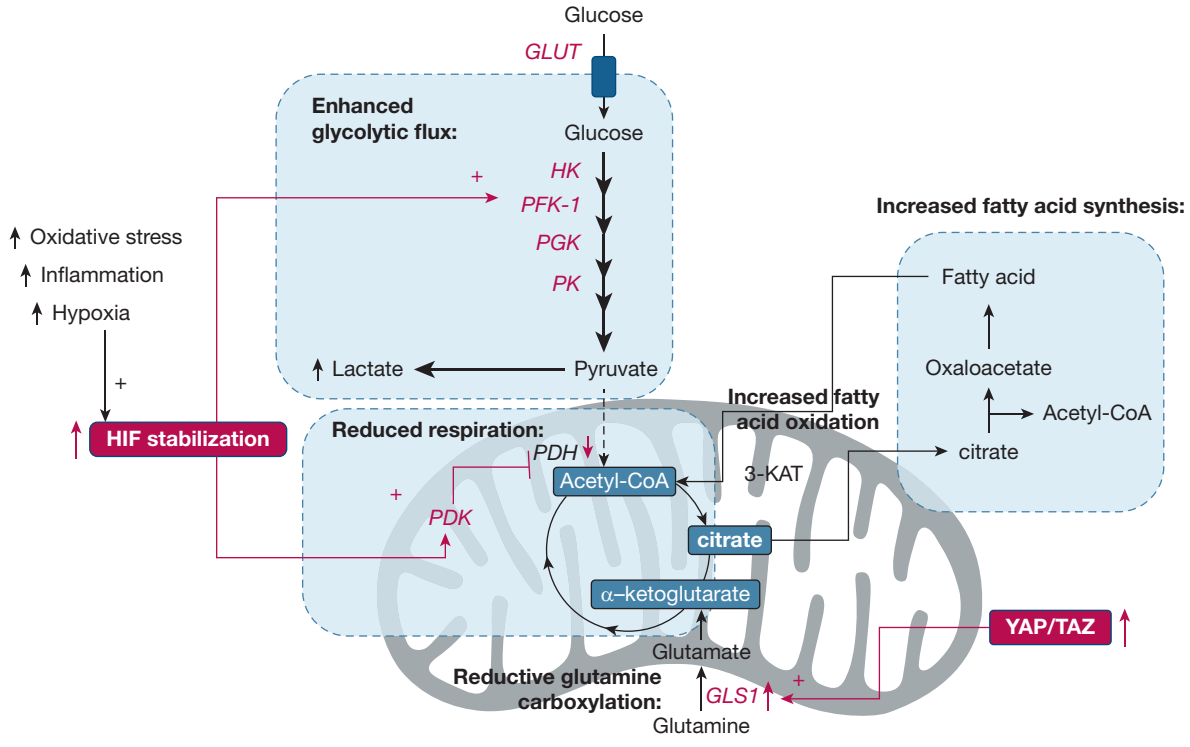

Figure 2 – *Overview of the dysfunctional metabolic pathways in pulmonary arterial hypertension pulmonary arterial smooth muscle cells. GLS1 = glutaminase1; GLUT = glucose transporter; HIF = hypoxia inducible factor; HK = hexokinase; KAT = lysine acetyltransferase; PDH = pyruvate dehydrogenase; PDK = pyruvate dehydrogenase kinase; PFK-1 = phosphofructokinase-1; PGK = phosphoglycerate kinase; PK = pyruvate kinase; TAZ = tafazzin; YAP = yess-associated protein.*

Altered Cell-Cell Communications in PAH: Interactions between resident pulmonary vascular contractile cells (PA-SMCs, adventitial fibroblasts, and pericytes) and ECs or immune cells are fundamental for several vascular functions, including maintenance of the diversity of the SMC phenotype and their intrinsic properties, and their adaptations in response to the local pulmonary arterial microenvironment.[27-29]

Alterations of pulmonary endothelium play a central role in the pathogenesis of PAH.[4,27,30] Pulmonary ECs in PAH indeed lose their ability to maintain an appropriate local balance between vasodilators (ie, impaired production of nitric oxide and prostacyclin) and vasoconstrictors (ie, enhanced production of endothelin-1 and serotonin), resulting in vasoconstriction of the pulmonary circulation. Elucidation of the dysfunctional mechanisms in these pathways has led to the development of all currently approved PAH therapies.[2] In addition to altered vasoreactivity in favor of pulmonary vasoconstriction, the pulmonary endothelium exhibits other functional and phenotypic alterations.[27] In PAH, the capacity of the pulmonary endothelium to form or maintain a functional vascular network through adequate secretion of the angiocrine factors required for vascular tissue homeostasis and repair is disrupted. In iPAH, dysfunctional pulmonary ECs are local and abnormal sources of various mediators that not only facilitate their own proliferation and survival,[31] but also influence the behavior of resident pulmonary vascular contractile cells.[19,27,30] In particular, these secreted mediators affect the interactions between pulmonary ECs and PA-SMCs and favor PA-SMC contraction, proliferation, and survival.[32-34] Therefore, a better understanding of signal transduction in the various pulmonary vascular cell types expressing high levels of specific RTKs (ie, platelet-derived growth factor receptor [PDGFR]-α, PDGFR-β, VEGFR1 [Flt-1], VEGFR2 [KDR], FGFR1, FGFR2) and non-RTKs (ie, Trk, Src, c-Abl, JAK) is central to selectively attenuating these specific growth-promoting signals without interfering with vital cellular processes.

The fact that inflammation very often precedes vascular remodeling in experimental PH and that a correlation between perivascular inflammation and the increase in pulmonary arterial wall thickness have been reported strongly support the importance of the interactions between pulmonary vascular contractile cells and immune and inflammatory cells.[5,28,29] However, it is still unclear how the crosstalk between inflammatory/immune cells and pulmonary wall components drives disease severity in PAH, limiting therapeutic options.

Vascular SMC Senescence: Similar to other senescent cells, senescent vascular SMCs are characterized not only by stable cell cycle arrest but also morphologic and metabolic changes, chromatin modifications associated with changes in the gene expression profile, and acquisition of a senescence-associated secretory phenotype (SASP), which is defined as abundant secretion of various proteins that influence both senescent cells and the local environment.[35] Senescent vascular SMCs are emerging as key drivers of chronic vascular inflammation and lung destruction in age-related respiratory disorders, including idiopathic pulmonary fibrosis and COPD.[36] Consistent with this notion, it has been shown in vitro that both the conditioned media and matrix of senescent cells facilitate the growth and migration of nonsenescent human PA-SMCs.[37-39] Interestingly, such pro-proliferative and promigratory effects were found to be partially dependent on the secretion of OPN, a multifunctional secreted integrin-binding glycoprotein associated with the SASP that can be immobilized in the ECM.[37] OPN is a pleiotropic factor involved in a range of physiological and pathologic processes, and its levels are known to increase with age.[40] Therefore, OPN can be considered to be an important mediator that promotes PH during ageing and during diseases associated with the accumulation of senescent PA-SMCs. Remodeled vessels in the lungs of patients with COPD were indeed found to be characterized by the presence of senescent PA-SMCs near actively dividing cells at sites of vessel wall hypertrophy, strongly supporting this idea.[38] However, although OPN levels are elevated in the serum and remodeled pulmonary vessel walls in patients with iPAH, the number of senescent cells found in the lungs of patients with iPAH is substantially lower than those found in the lungs of patients with COPD.[37] Several intrinsic and extrinsic factors can activate the cellular senescence program, including telomere damage, DNA damage, and mitochondrial dysfunction. Although several signaling pathways are involved in the cellular senescence program, the accumulation of p53 and $p16^{Ink4a}$ plays a central role.[41] Indeed, it has been shown that exposure of human PA-SMCs to the MDM2 antagonist and p53 activator nutlin-3a results in the arrest of cell growth, with the induction of senescence but not apoptosis.[42] Nutlin-3a has been shown to exert beneficial effects against the development of PH induced by chronic

hypoxia in wild-type mice, but not $p53^{-/-}$ or $p21^{-/-}$ mice. Therefore, nutlin-3a may hold promise as a prosenescence treatment that targets PA-SMCs during PH progression.[42] However, a better understanding of the mechanisms and functions of senescent cells is essential because cellular senescence impairs the reversibility of the pulmonary vascular remodeling associated with PAH.[36] Indeed, the SASP of senescent pulmonary ECs stimulates cell proliferation/survival and induces a phenotypic switch of SMCs in culture[39] and has the ability to activate crucial pathways (eg, mammalian target of rapamycin pathway).[32,33,43] Consistent with this notion, the development of new drugs that allow the specific elimination of senescent cells, termed senolysis, also holds promise to delay or reverse experimental PH.[32,33,42] This knowledge could be helpful in identifying which features of the senescent cells can be targeted without negatively affecting normal cells.

Therapeutic Options to Reverse Vascular Contractile Cell Dysfunction in PAH

Identifying an effective therapeutic agent that selectively targets the pulmonary smooth muscle-related dysfunction that occurs in PAH, with minimal side effects, is a considerable challenge. Nonetheless there are a variety of drugs that are currently used or under investigation (Table 1). These various molecules exert their actions on pulmonary smooth muscle but, to a greater or lesser extent, also interfere with other disease components (eg, pulmonary endothelial dysfunction, persistent inflammation, imbalances in cellular apoptosis/proliferation, increased oxidative stress).

Targeting Hyperproliferation and Vascular Remodeling

Over the last several years, special attention has been devoted to the identification of strategies that can block growth-promoting signals to normalize the exaggerated migration, proliferation, and survival of pulmonary vascular contractile cells. Several agents, including certain tyrosine kinase inhibitors, have shown beneficial effects in rodent models.[4,13,18-20] However, enthusiasm concerning these specific antiremodeling strategies has been tempered by the fact that imatinib is poorly tolerated by patients with PAH and associated with frequently reported side effects and that dasatinib induces endothelial dysfunction and PAH in humans.[44-47] Because of these serious side effects observed with orally administered tyrosine kinase inhibitors, new formulations administered by inhalation are currently in clinical development for PAH, including for imatinib[48] and seralutinib.[49,50] Several preclinical studies have also focused on metabolic pathways related to oxidative stress and energy metabolism. Consistent with findings obtained in experimental PH models, a clinical trial found that dichloroacetate, an inhibitor of the mitochondrial enzyme PDK, improves hemodynamics and functional capacity in a subset of patients that do not carry functional variants of *SIRT3* and *UCP2* (encoding sirtuin-3 and uncoupling protein 2, respectively).[21] Other affected metabolic pathways are also present in PAH, including alterations in glucose homeostasis and carnitine, fatty acid, and glutamate metabolism, and the potential therapeutical efficacy of several medications targeting these metabolic changes is the subject of ongoing research (ie, metformin, thiazolidinediones, inhibitors of fatty acid β-oxidation). In this context, dysregulation in the TGF-β/activin/nodal and BMP/GDF branches (NCT03496207),[16,17] and the presence of chronic inflammation,[4,5] are strongly suspected to play critical roles.

As recently reviewed,[5] numerous investigators are actively studying the therapeutic targeting of the various mechanisms by which inflammation contributes to PAH pulmonary vascular remodeling in PAH with various compounds with antiinflammatory and/or immunomodulatory activity. Several clinical trials are indeed exploring the impact of various antiinflammatory agents in PAH. These include, notably, rituximab,[51] a chimeric antihuman CD20 antibody (NCT01086540), and anakinra,[52] an IL-1 receptor antagonist (NCT03057028). Although results with the LTB4 blocker ubenimex and the IL-6 receptor antibody tocilizumab were unsatisfactory,[53,54] they contribute to our understanding of the complexity of the inflammatory component in PAH, which can compensate for the inhibited component to maintain the activity of key downstream circuits, even in the presence of drugs.

As previously mentioned, the well-documented role of the dynamic remodeling of the ECM on the accumulation of PA-SMCs/ECs suggests the possibility of combining current PAH-targeted therapies with a specific agent that can limit such maladapted ECM remodeling of the pulmonary vascular wall.[4,55] Elafin, an endogenous serine protease inhibitor, is being developed for the treatment of PAH.[56] Pulmonary vascular cells derived from patients with PAH show a high capacity to secrete various components of the ECM and locally change its quality, quantity, and distribution. Consequently, the pulmonary vascular cells in PAH

TABLE 1] Current Drug Candidates Under Clinical Investigation in Pulmonary Arterial Hypertension

Drug Candidate	Clinical Trial	Mechanism of Action and Target
ABI-009	NCT02587325 (recruiting)	mTOR inhibitor
Acetazolamide	NCT02755259 (completed)	Carbonic anhydrase inhibitor
Albuterol	NCT03270332 (completed)	β2-adrenergic receptor agonist
Anastrazole	NCT03229499 (recruiting)	Aromatase inhibitor
Apabetalone	NCT03655704 (recruiting)	BDR4 inhibitor (BET transcriptional regulators)
Bardoxolone methyl	NCT02036970 (completed)	Nrf2/NF-κB signaling pathway
CXA10 (10-nitro-9(E)-octadec-9-enoic acid)	NCT03449524 (terminated)	Nitrated fatty acid
Dichloroacetate	NCT01083524 (completed)	Pyruvate dehydrogenase inhibitor
Elafin	NCT03522935 (completed)	Elastase inhibitor
Escitalopram Fluoxetine	NCT00190333 (completed) NCT03638908 (completed)	Serotonin transporter inhibitor
Imatinib	NCT00902174 (completed) NCT04903730 (recruiting)	PDGFR-β/α inhibitor
Metformin	NCT03629340 (Active, not recruiting)	Antidiabetic drug
Olaparib	NCT03782818 (recruiting)	PARP1 inhibitor/DNA damage inhibitor
Pemziviptadil (PB1046)	NCT03795428 (recruiting)	VIP analog
Ranolazine	NCT01757808 (completed)	Sodium channel blocker
Rituximab	NCT01086540 (completed)	Anti-CD20
Rodatristat ethyl	NCT04712669 (recruiting)	TPH-1 inhibitor
Seralutinib (GB002)	NCT03926793 (completed) NCT04456998 (recruiting)	PDGFR kinase inhibitor
Sorafenib	NCT00452218 (completed)	Raf-1 kinase inhibitor (VEGF inhibitor)
Sotatercept	NCT03496207 (Active, not recruiting)	Activation of BMPRII signaling (ligand trap)
Tacrolimus	NCT01647945 (completed)	Activation of BMPRII signaling
Tocilizumab	NCT02676947 (completed)	IL-6 receptor antagonist
Trimetazidine	NCT03273387 (completed)	3-ketoacyl-CoA thiolase inhibitor
Ubenimex	NCT02664558 (completed)	Leukotriene B4 inhibitor
Zamicastat (BIA 5-1058)	NCT04316143 (recruiting)	Dopamine B-hydroxylase inhibitor

BET = bromodomain and extra terminal domain; BMPRII = bone morphogenetic protein receptor type II; Brd4 = bromodomain-containing protein 4; CD = cluster of differentiation; IL = interleukin; mTOR = mammalian target of rapamycin; NF-κB = nuclear factor-kappa B; Nrf2 = nuclear factor erythroid 2–related factor 2; PARP1 = poly (ADP-ribose) polymerase 1; PDGFR = platelet-derived growth factor receptor; TPH-1 = tryptophane hdrolase-1; VEGF = vascular endothelial growth factor; VIP = vasoactive intestinal peptide.

have the capacity to modulate vessel stiffness and create a permissive pericellular/extracellular environment for the progression of pulmonary vascular remodeling. Along with the release of ECM fragments, the excessive release of growth factors and other molecules incorporated into the ECM and the exposure of functionally cryptic sites that collectively result in the maladapted ECM remodeling observed in PAH profoundly influence cell motility, proliferation, apoptosis, and differentiation. Matrix metalloproteinase-10, overexpressed in the serum and pulmonary arteries of patients with scleroderma-associated PAH, represents a putative treatment target because its blockade reduces PH in a murine model.[57] However, our current understanding of the organization and stoichiometry of the various vascular ECM proteins and the mutualistic relationships in the networks between proteases and inhibitors during PAH progression is incomplete, and a large number of questions remain to be answered.

BMP/TGF-β Receptor Signaling Pathway as a Therapeutic Target

Signaling pathways involving members of the TGF-β superfamily, their various ligands, and transcriptional

coregulators coordinate the regulation of gene expression that is central for the development and maintenance of various tissues and organs, especially for the pulmonary vascular endothelium, in which BMPRII and ALK1 are highly expressed.[16,58] As recently reviewed,[16] TGF-β superfamily signaling has indeed emerged as a central actor in the pathogenesis of PAH. It is well established that decreased activity of the BMP-GDF branch, which acts through the Smad 1/5/8 signaling pathway, plays a critical role in the predisposition to PAH and disease progression; however, the cause remains unclear. Conversely, overactivation of the TGF-β-activin-nodal branch, acting through the canonical Smad 2/3 signaling pathway, has been described in experimental and human PAH.[16,58] The ligands of the TGF-β-activin-nodal branch can antagonize the ligands of the BMP/GDF branch because of ligand-receptor affinity, which hinders the actions of pSmad1/5/8 in SMCs.[34] Yang et al[59] showed that the overexpression of mutant BMPRII in pulmonary ECs induces apoptosis and prompts the release of TGF-β. It has also been reported that TGF-β1 released from apoptotic ECs facilitates PA-SMC proliferation,[60] whereas the blockade of activin A and TGF-β reduces their proliferation.[61] It has also been documented that the enhanced responsiveness of PA-SMCs to TGF-β1 is associated with excessive secretion of proinflammatory cytokines, namely IL-6 and IL-8.[62] A study comparing patients with iPAH with control subjects found higher levels of TGF-β in the lungs and sera of patients with iPAH, with increased phosphorylation of Smad1/5/8 after treating ECs from these patients with increasing doses of TGF-β. Conjointly, the medium from these cells stimulated PA-SMC growth.[63] Zabini et al[64] demonstrated that chronic stimulation with TGF-β of both pulmonary ECs and PA-SMCs induces the downregulation of Smad3 and increases the proliferation and migration of these cells. This corroborates the proven fact that the TGF-β/Smad2/Smad3 axis is impaired in experimental PH and iPAH.[16] Interestingly, Yu et al[65] described a compensatory mechanism between certain receptors of the TGF-β superfamily in PA-SMCs when BMPRII is lost. In their model, the signaling of certain BMP ligands, namely BMP6 and BMP7, was elevated in deficient cells because of a rescue by ActRIIA, which was shown by the upregulation of Id1 gene expression. This interplay between TGF-β superfamily members is partly responsible for the excessive vascular cell accumulation and the consequential obliteration of the pulmonary artery lumen observed in PAH. Ogo et al[66] investigated the role of prostacyclin analogs, universally used in the treatment of PAH, in TGF-β signaling. They demonstrated an inhibitory effect of such molecules on TGF-β1 activity in vivo in a monocrotaline-induced rat model of PAH and in vitro in SMCs of mice with *BMPR2* mutations. Macitentan, a dual endothelin receptor antagonist, another new drug class in PAH treatment, is also effective against experimental PH induced by the overexpression of TGF-β.[67] Additionally, the inhibition of ALK5, a TGF-β type I receptor, by SD208 reduced vascular remodeling in monocrotaline-induced PH.[68] In another report, the blockade of activin A and TGF-β reduced the proliferation of SMCs from patients with PAH.[62]

Among the numerous approaches that aim to restore the interactions and crosstalk between the TGF-β/activin/nodal and BMP/GDF branches (eg, FK506 [tacrolimus], FK520 [ascomycin], rapamycin, ataluren [PTC124], 4-phenylbutyric acid [4PBA], the administration of recombinant BMP-9 protein),[16] the use of ligand-trap molecules is the most promising. In the phase 2 PULSAR trial, treatment with the human ActRIIA-Fc, sotatercept, for 24 weeks resulted in a greater reduction in pulmonary vascular resistance than placebo in patients with PAH on stable background therapies (NCT03496207).[17]

Limiting Progenitor Cell Recruitment/Differentiation and Endothelial-Mesenchymal Transition

Over the last several years, advances in our knowledge have also enabled the demonstration that a variety of circulating and lung-resident progenitors also contribute to the abnormal narrowing of small vessels in PAH and as sources of smooth muscle-like cells.[4,12,69–75] Various pulmonary resident progenitors positive for PW-1 and other stem cell markers, including Sca-1, c-kit, PDGFR-α, CD34, and/or other mesenchymal markers, have indeed been shown to accumulate in the tissue parenchyma and perivascular regions in remodeled pulmonary vessels.[71] In addition, CD146-, NG2-, 3G5-, and PDGFR-β-positive perivascular cells are also well-known SMC progenitor cells.[12,70,72–74] In human PAH, the demonstration that pulmonary arterioles are two to three times more highly covered by pericytes[70] has paved the way for lineage tracing studies, showing that such resident progenitor vascular cells foster the onset and maintenance of PAH structural changes in blood lung vessels.[72,73,75] In addition to their role in vascular cell accumulation and blood vessel formation, pericytes are also known to be central regulators in vascular development, stabilization, maturation, and leucocyte extravasation. Interestingly, attenuation of established PH in an animal model was demonstrated via the neutralization of C-X-C motif chemokine ligand-12

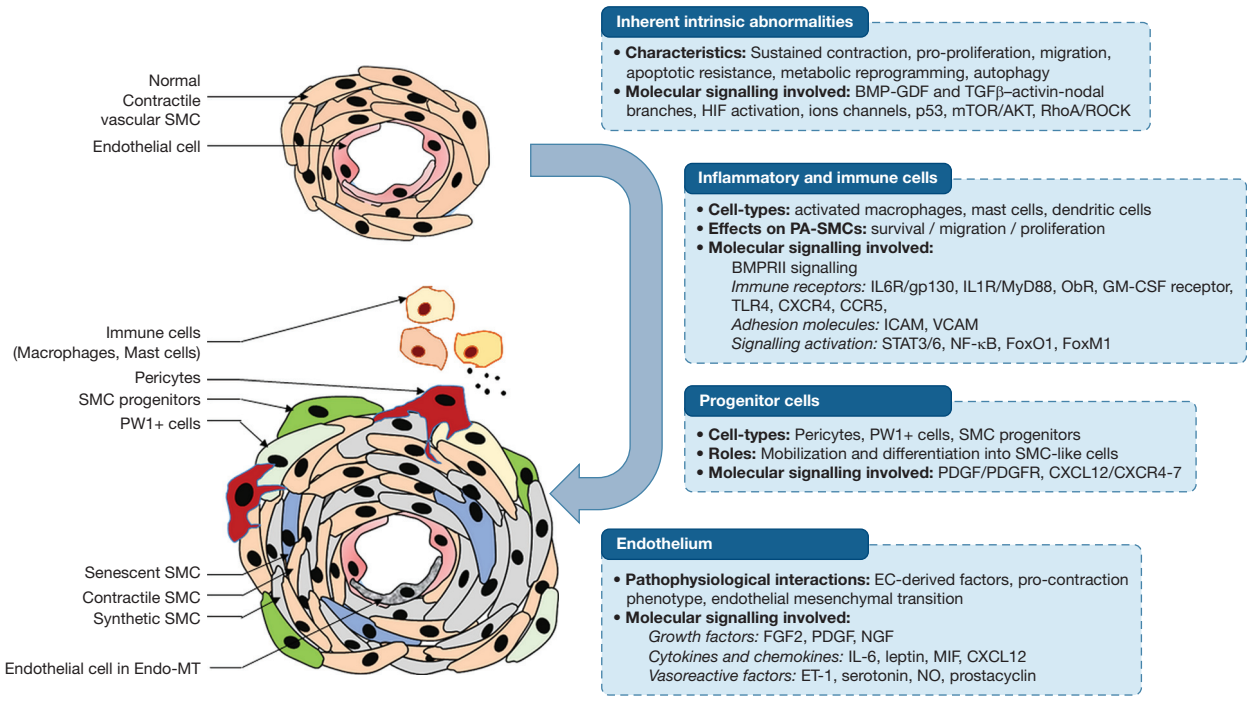

Figure 3 – *Mechanisms underlying changes in the functional properties of the pulmonary vascular contractile cells in pulmonary arterial hypertension. AKT = protein kinase B; BMP = bone morphogenetic protein; CCR5 = C-C chemokine receptor type 5; CXCL12 = C-X-C motif chemokine ligand 12; CXCR4 = C-X-C motif chemokine receptor 4; CXCR4-7 = C-X-C motif chemokine receptor 4 and 7; EC = endothelial cell; Endo-MT = endothelial-mesenchymal transition; ET-1 = endothelin-1; FGF2 = fibroblast growth factor; FoxM1 = forkhead box M1; FoxO1 = forkhead box O1; GDF = growth differentiation factor; GM-CSF = granulocyte-macrophage colony-stimulating factor; gp130 = glycoprotein 130; HIF = hypoxia inducible factor; ICAM = intercellular adhesion molecule; MIF = macrophage migration inhibitory factor; mTOR = mammalian target of rapamycin; MyD88 = myeloid differentiation primary response 88; NF-κB = nuclear factor-kappa B; NGF = nerve growth factor; NO = nitric oxide; ObR = leptin receptor; PA-SMC = pulmonary artery smooth muscle cell; PDGF = platelet-derived growth factor; PDGFR = platelet-derived growth factor receptor; PW1 = gene/paternally expressed gene 3 (PEG3); RhoA = Ras homolog family member A; ROCK = Rho-associated protein kinase; SMC = smooth muscle cell; STAT3/6 = signal transducer and activator of transcription; TGFβ = transforming growth factor beta; TLR4 = toll-like receptor 4; VCAM = vascular cell adhesion molecule.*

(CXCL12), which decreased vascular pericyte coverage, PA-SMC proliferation, and macrophage infiltration in the lungs.[12,69] Consistent with these results, mice deficient in CXCL12 or treated with a CXC receptor (CXCR)-4 inhibitor (AMD3100) are protected against PH induced by chronic hypoxia.[72] Comparative analyses of the in vitro cellular fate of freshly cultured human pericytes derived from PAH and control lung specimens have further accentuated the importance of this specific pulmonary vascular cell type in PAH. Relative to donor control cells, cultured PAH pulmonary pericytes have been found to overexpress PDK-4, a key metabolic regulator that contributes to an influential shift from the use of glucose to fatty acids as a major energy source, and CXCR-7, which binds with high affinity to CXCL12 and the type II TGF-β receptor.[12,74] In addition, they lose their ability to secrete Wnt5a.[74] In light of these discoveries, pericytes and other resident or circulating progenitors could become candidates for better tailored therapeutic treatment, and a powerful tool for tissue engineering and regeneration. Nevertheless, there is still confusion concerning the identity, ontogeny, and progeny of the various progenitor cells present in the lungs, and dissection of their precise roles in the progression of the pulmonary vascular remodeling in PAH will require further work.

Because pulmonary ECs can go through endothelial-mesenchymal transition (EndoMT) to transform into mesenchymal/smooth muscle-like cells in various cardiovascular diseases, including in PAH,[76–79] drugs targeting EndoMT may also be a promising therapy against PAH. However, it remains an open question as to whether these EndoMT-transformed cells or cells derived from circulating and lung-resident progenitors exhibit similar phenotypic features and biological functions as resident PA-SMCs or other contractile cells (eg, adventitial fibroblasts, pericytes). In addition, whether their specific elimination may delay, prevent, or reverse the disease is still unknown.

Conclusions

Currently available PAH therapies predominantly target endothelial dysfunction and improve symptoms and

survival. However, despite major advances in our understanding of PAH over the last two decades, no available treatments reverse the abnormal pulmonary vascular proliferation and remodeling that lead to right ventricular failure. There is now a considerable need to develop innovative therapies that target the cellular and molecular mechanisms that regulate the progressive obstructive vascular remodeling in PAH (Fig 3).

Acknowledgments

Authors contributions: All authors are guarantors of the content of the manuscript. All authors contributed substantially to the writing of the manuscript, have read and approve the manuscript.

Financial/nonfinancial disclosures: The authors have reported to *CHEST* the following: M. H. reports grants and personal fees from Acceleron, Bayer, Merck, and Janssen; and personal fees from GSK, outside the submitted work. C. G. reports grants from Acceleron, ShouTi, and Janssen, and grants and personal fees from Merck, outside the submitted work. None declared (B. L., N. B., A. H., L. T.).

Role of sponsors: The sponsor had no role in the design of the study, the collection and analysis of the data, or the preparation of the manuscript.

Other contributions: The research laboratory of C. Guignabert and M. Humbert is supported by grants from the French National Institute for Health and Medical Research (INSERM), the Université Paris-Saclay, and the French National Agency for Research (ANR) grant n° ANR-16-CE17-0014 (TAMIRAH) and ANR-17-CE14-0011 (COVER), and in part by the Département Hospitalo-Universitaire (DHU) Thorax Innovation (TORINO), the Assistance Publique-Hôpitaux de Paris (AP-HP), Service de Pneumologie, Centre de Référence de l'Hypertension Pulmonaire Sévère, the Marie Lannelongue Hospital - Groupe Hospitalier Paris Saint Joseph, the French PAH patient association (HTAP France), the Fonds de Recherche en Santé Respiratoire (FRSR) and the Fondation du Souffle (FdS).

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