



Molecular mechanisms involved in pulmonary arterial hypertension development

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Abstract

Pulmonary arterial hypertension (PAH) is an elevation in pulmonary arterial pressure, characterized by symptoms of dyspnea, chest pain, decrease in exercise tolerance-fatigue, syncope and, if untreated, PAH leads to right heart failure.

In PAH, there is an imbalance between mediators of vasodilation and vasoconstriction (e.g. nitric oxide and prostacycline – potent vasodilators, platelet inhibitor and antimitogens are decreased in PAH, while thromboxane, vasoconstrictor and platelet activator is increased in PAH, resulting in smooth muscle hypertrophy of small vessels, adventitial and intimal proliferation, and plexiform vascular lesions with vascular thrombosis). Standard diagnostic procedures for PAH include physical examination, pulmonary function testing, radiographic imaging, transthoracic echocardiography, right heart catheterization. Current drugs include synthetic prostanoids (iloprost, epoprostenil, beraprost, treprostinil) – vasodilators and anti-platelet agents. Phosphodiesterase-5 inhibitors decrease the breakdown of cGMP, increasing its intracellular levels, leukotriene receptor antagonist, – zafirlukast, decreases pulmonary arterial and venous pressure. Endothelin receptor blockers, bosentan, decrease pulmonary vascular resistance and improve results of functional tests. Other treatments are: anticoagulants, calcium-channel blockers, positive airway pressure therapy for obstructive sleep apnea, or oxygen for hypoxemia, and surgery. In conclusion, although there are some promising drugs in therapy of PAH, there is a need to develop new ones, together with surgical approaches, in order to increase the survival of patients with PAH. Gene and cell therapy could be expected as future perspectives.

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a disease that causes notable morbidity and mortality and is clinically demonstrated by increasing pulmonary arterial pressure (1). The incidence of PAH is 2,4–7,6/per million, with the prevalence of 15–52 patients / per million, affecting twice as many females as males (1, 2). The epidemiological data show that the one-, three-, and five-year survival rates were 68%, 48%, and 34%, respectively; with the progress of modern therapeutic agents, these survival rates have improved to 86%, 69%, and 61%, respectively (1).

PAH had been classified as a primary (idiopathic) or secondary form (3). The recent World Health Organization classification of pulmonary hypertension includes five categories: idiopathic PAH is the most

prevalent form of PAH (46.2% of patients). Familial forms of PAH caused by mutations in bone morphogenetic protein receptor-2 or activin, like kinase 1, account only for 2.7% of cases (3). Other etiologies of PAH include: connective tissue disorders (25.3%), congenital heart disease (9.8%), liver disease (5.3%), human immunodeficiency virus infection (1.9%), and medication usage (5.3%), pulmonary veno-occlusive disease and pulmonary capillary hemangiomatosis, for less than 0.5% of cases (3). Separate classification includes the following diseases: pulmonary hypertension due to left-sided heart disease, hypoxemic lung disease, and thromboembolic disorders (4). A miscellaneous class includes sarcoidosis, pulmonary Langerhans cell histiocytosis, lymphangiomatosis, fibrosing mediastinitis, and other neoplasms that compress the pulmonary arteries (4). The term PAH comprises the primary forms as well as manifestations resulting from other diseases, such as collagen vascular disease, hemoglobinopathies, congenital cardiovascular disease with systemic-to-pulmonary shunt, human immunodeficiency virus infection, portal hypertension, drugs and toxin, persistent pulmonary hypertension of the newborns and myeloproliferative disorder (4). The symptoms of PAH are: dyspnea (60%), and fatigue (19%), chest pain (7%), syncope (8%), lower extremity edema (3%), and palpitations (5%). The diagnostic procedure includes: physical examination, pulmonary function testing, radiographic imaging, transthoracic echocardiography, right heart catheterization-gold standard for PAH diagnosis and differentiation between PAH and pulmonary venous hypertension (2).

The goal of this review is to present current knowledge on PAH etiopathogenesis in terms of molecular mechanisms involved, main clinical features and diagnosis, and to emphasize the therapeutical approach and novelties in drug treatment.

MOLECULAR PATHWAYS CONTRIBUTING TO ETIOPATHOGENESIS OF PAH

Development of PAH includes the complex interaction of vascular effectors at all anatomic levels of the arterial wall (1). Vasoconstriction, thrombosis, and inflammation are major pathophysiological events leading to vessel wall remodeling and cellular hyperproliferation. Histologic features in PAH occur at the level of the small peripheral pulmonary arteries. These include intimal fibrosis, proliferation of vascular smooth muscle, and pulmonary arterial occlusion (4). End stage disease involves the formation of a vessel neointima (increased deposition of extracellular matrix and myofibroblasts) (4).

Vascular endothelial cell plays a crucial role in the regulation of the pulmonary vascular tone and in the maintenance of the barrier function and integrity of the alveolar-capillary membrane (4). It also plays an important role in coagulation, fibrinolysis, and angiogenesis and participates in inflammatory response.

The endothelium is a major detector of injurious stimuli such as: inflammation, hypoxia, toxins, shear stress (5).

An imbalance of secreted vasoactive factors and vascular remodeling via pathologic cellular processes, such as excessive cell proliferation, vasoconstriction, and thrombosis, associated with more specific inflammatory responses and angiogenesis are presented in PAH (6). For example, in PAH, there is excessive production of endothelin and thromboxane, both of which are vasoconstrictors. As a result, there is a deficit of the vasodilators, such as prostacyclin and nitric oxide (4). In addition, dysregulation of circulating and resident progenitor cells may also contribute to PAH (6).

GENETIC BACKGROUND OF PAH

Some forms of PAH, as previously mentioned, have strong genetic background. For example, mutations of transforming growth factor- β receptor (TGF- β receptor) superfamily are linked to PAH and likely play a causative role in the development of disease (7). Patients with hereditary hemorrhagic teleangiectasia and idiopathic PAH have specific mutations in ALK 1 or endoglin genes, encoding for two such members of the TGF- β receptor super family (8). A more prevalent cohort of patients carries mutations in another gene, namely, the bone morphogenetic protein receptor type 2 (BMPR2) gene which encodes for BMPR-II (8). There are more than 140 mutations in BMPR2 that have been reported in patients with familial PAH (8). The absence of BMPR 2 mutations in some familial cohorts and in most sporadic cases indicates that unidentified genetic mutations can also predispose to the development of PAH (8). BMPR-II has a complex mechanism of action, it functions as a receptor and it participates in a number of complex intracellular pathways that lead to regulation of gene transcription (8). BMPR-II is expressed in the pulmonary endothelium, medial smooth muscle cells, and macrophages (8). The failure of BMP ligands to exert suppressive and protective effects on vascular smooth muscle and on the endothelium leads to vascular remodeling and proliferation (9). Dysregulation of the BMP signaling pathway may result in PAH. The fundamental analysis of the mechanisms of the genetic predisposition to PAH is crucial for interpretation of pathogenesis of PAH.

Another signaling pathway, the serotonin (5-hydroxytryptamine or 5-HT) signaling pathway, has been investigated as a potential causative factor in PAH (10). Serotonin acts as a vasoconstrictor and mitogen that promotes smooth muscle hyperplasia and hypertrophy (10). After secretion from platelet granules, serotonin binds G-protein-coupled serotonin receptors present on pulmonary artery smooth muscle cells (10). The receptor activation leads to a decrease in adenylyl cyclase and cAMP, causing an increase in contraction (10). The cell-surface serotonin transporter (5-HTT) allows for transport of extracellular serotonin into the cytoplasm of smooth muscle cells, activating cellular proliferation in two ways: di-

rectly through the action of serotonin or indirectly via potential pleiotropic mechanisms (11). Also, serotonin can inhibit BMP signaling via modulation of downstream Smad proteins and can regulate the angiopoietin -1/Tie2 signaling pathway, another pathogenic contributor – modulating the signaling of BMPR-II via inhibition of endothelial expression of one of its dimerized partners, BMPR-I A (12). Primary PAH is characterized by increased pulmonary expression of serotonin receptors and by elevated plasma levels of serotonin (11).

OTHER FACTORS CONTRIBUTING TO THE DEVELOPMENT OF PAH

Exogenous influences include chronic hypoxia, hemoglobinopathies, autoimmune vascular disease, viral infections, and congenital heart disease with systemic to pulmonary shunt (13). Hemoglobinopathies (thalassemias and sickle cell anemia) could involve PAH (13). Hemolysis in hemoglobinopathies leads to the destruction of bioactive nitric oxide by free hemoglobin or reactive oxygen species (13). An inflammatory and proliferative cascade may ensue with culmination in PAH (13). PAH could complicate autoimmune diseases, especially in the setting of the CREST (Calcinosis, usually in fingers; Raynaud's; loss of muscle control of the Esophagus; Sclerodactyly and Telangiectasia) a variant of limited systemic sclerosis and in mixed connective tissue diseases such as systemic lupus erythematosus and rheumatoid arthritis. In the setting of pulmonary fibrosis and hypoxia, inflammation and deposition of extracellular matrix may increase vasoconstriction, proliferation, and vessel remodeling (14).

The human immunodeficiency virus (HIV) infection and pulmonary hypertension has been noted in approximately 0.5% of all patients with HIV infection (6–12 times higher than the general population) (15). As a result, there is an infection of smooth muscle cells with subsequent dysregulation of proliferation, imbalance of vascular mitogens in response to systemic HIV infection, and endothelial injury precipitated by HIV-infected T cells (15). Human herpes virus 8 (HHV-8) is highly associated with HIV infection, and may play a role in PAH development with progression to plexiform lesions (15).

Increased flow through the pulmonary circulation may also play a role in development of PAH (16). Several congenital heart diseases with functional systemic – pulmonary shunts, such as unrestricted ventricular septal defects (VSD) and large patent ductus arteriosus (PDA), lead to pulmonary vascular remodeling and PAH during childhood (Eisenmenger's syndrome) (16). Atrial septal defects (ASD) with systemic – to pulmonary shunts lead to PAH over time. Endothelial cells recognize turbulent flow and cyclic strain leading to transduction of intracellular signals and the modulation of a wide variety of phenotypic changes (16). Laminar flow induces vasoprotection, while turbulent flow gives rise to a pro-inflammatory and thrombotic state. PAH has been detected in patients with other clinical syndromes such as idio-

pathic thrombocythemia, thrombocytosis, chronic myelodysplastic syndromes, portal hypertension, neonates with failure of the fetal-to-neonatal circulatory transition, and in patients exposed to stimulants of the central nervous system (methamphetamines and cocaine) (17).

Histopathologic processes in later stages of the disease include thrombosis, vasoconstriction and cellular proliferation (17). As a result of dysbalance of vascular effectors controlling vasodilatation and vasoconstriction, growth suppressors and growth factors, and pro-versus anti-thrombotic mediators, the progression of the disease occurs (17). Nitric oxide (NO) is a potent pulmonary arterial vasodilator and a direct inhibitor of platelet activation and vascular smooth muscle cell proliferation (18). The synthesis of NO is mediated by a family of NO synthase enzymes (NOS) (18). The endothelial isoform (eNOS) is regulated by a number of vasoactive factors and physiologic stimuli (hypoxia, oxidative stress and inflammation) (18). Carbon monoxide (CO) and hydrogen sulfide (H₂S) are endogenously produced gaseous vasodilators, the deficiencies of which may promote the development of PAH (19). H₂S acts as a vasodilator and inhibitor of vessel wall proliferation which can protect against the development of PAH in rat models (20). Prostacyclin (PGI₂), a prostanoid metabolized from endogenous arachidonic acid through the cyclooxygenase (COX) pathway, is a potent vasodilator that has been identified as one of the most effective drugs for the treatment of pulmonary arterial hypertension (21). The biological functions of prostacyclin in the pulmonary circulation are mediated by a specific cell-surface receptor. This receptor belongs to the G-protein coupled receptor (GPCR) (22). The binding of prostacyclin to the receptor results in activation of the G-protein and increases intracellular cAMP which activates protein kinase A (22). This produces inhibition of platelet aggregation, relaxation of smooth muscle, and vasodilation of pulmonary arteries (20). Patients with PAH have decreased levels of prostacyclin synthase in small and medium pulmonary arteries. Another arachidonic acid metabolite, thromboxane A₂, is involved in thrombosis, vasoconstriction and vessel wall proliferation (20). Thromboxane A₂ increases vasoconstriction and activates platelets (20).

Endothelin-1 (ET-1) is expressed by pulmonary endothelial cells and acts as a pulmonary arterial vasoconstrictor and a mitogen of pulmonary smooth muscle cells (20). As a result of its activity (vasoconstriction, remodeling, mitogenesis), there are significant hemodynamic changes that lead to PAH (23). The human endothelin family (ET) consists of three 21-amino acid isopeptides: ET-1, ET-2, and ET-3 (24). Only ET-1 plays important physiological and pathophysiological role in the regulation of vascular tone. ET-1 is released principally from endothelial cells. Most of its effects are paracrine, the most striking is its extreme and long-lasting vasoconstrictor action (24). ET-1 can induce hypertrophy and hyperplasia in various cell types, fibroblast proliferation, extracellular matrix production, inflammation, and neuro-humoral stimulation (24). However, depend-

ing on the receptor type that ET-1 binds to, it stimulates other local mediators of vascular tone (nitric oxide (NO), prostacyclins, and platelet-activating factors) (Figure 1). There is a number of pathological conditions that could elevate the levels of circulating ET-1 (atherosclerosis, arterial hypertension, heart failure, PAH) (25). ET-1 acts through two receptor subtypes – ETA and ETB. In the vasculature, ETA receptors are located on smooth muscle cells and fibroblasts, whereas ETB receptors are localized on endothelial cells and, to a lesser extent, on smooth muscle cells, fibroblasts, and macrophages. Activation of ETA receptors mediates vasoconstriction, proliferation, hypertrophy, cell migration, and fibrosis, whereas activation of endothelial ETB receptors stimulates the release of potent vasodilators (NO and prostacyclin) which exhibit anti-proliferative properties, and prevents apoptosis (25).

Dysregulation of vasoactive intestinal peptide (VIP) can be involved in the pathogenesis of PAH (23). VIP is pulmonary vasodilator and it is decreased in PAH, both in serum and lung tissue (26). Pulmonary arterial and pulmonary vascular resistances decrease after treatment with VIP (26).

There are some other vascular elements that could initiate pulmonary vascular disease (dysfunction of adventitial fibroblasts, components of the extracellular matrix) (5). Vascular endothelial growth factor (VEGF) is a central growth and survival factor for the endothelial cell and exerts a variety of physiological actions in the lungs, where particularly high levels of VEGF are expressed (4). VEGF acts as a cell mitogen and angiogenic factor. Apoptosis followed by a selection of apoptosis-resistant cells may play a pathogenic role in PAH, triggered by VEGF receptor 2 (VEGFR-2) blockade.

The modulation of voltage-gated potassium channels (Kv) may also represent a pathogenic mechanism of PAH (27). Kv channels are inhibited in the smooth muscle cells in the pulmonary arterial tree in response to

hypoxia (27). Depolarization leads to the opening of voltage-gated calcium channels and the initiation of intracellular signaling cascades promoting vasoconstriction and proliferation and inhibiting apoptosis (227). This activity is regulated by serotonin, nitric oxide and thromboxane A₂ (27). The Kv pathway may represent a root of regulation in the pathogenesis of PAH.

Angiopoietin-1 is an angiogenic factor which is included in regulation of the vascular effectors of PAH (27). It is produced by smooth muscle cells and pericytes: it binds the Tie2 receptor which then activates smooth muscle migration and proliferation (27). Angiopoietin-1 can stimulate pulmonary arterial endothelial cells to secrete mitogenic factors (serotonin and endothelin-1) (28). It represents a major connection between BMP and serotonergic pathways.

The actions of caveolae and its protein caveolin-1 (CAV-1) may also be a possible regulatory pathway of the development of PAH (28). Calveolae are flask-shaped invaginations found on the surface of the plasma membrane in variety of vascular cell types (pulmonary endothelium, vascular smooth muscle cells, and fibroblasts) (28). CAV-1 is depleted in plexiform lesions and muscularized pulmonary arterioles from patients with PAH (28). The pathogenesis of PAH may include severe inflammatory reaction mediated by interleukin-1 β , transforming growth factor- β 1, bradykinin, monocyte chemoattractant protein-1, fractalkine, RANTES and leukotrienes, among others. 5-lipoxygenase (5-LO) regulates the synthesis of leukotrienes (28). Vascular-specific serine elastase activity has been involved in the pathogenesis of PAH via regulation of the remodeling response in the extracellular matrix (28). Serine elastases are secreted into the extracellular space to activate matrix metalloproteinases (MMP) and to inhibit tissue inhibitors of MMP (TIMP) (28). Both MMP and elastases degrade most components of the extracellular matrix, leading to an upregulation of fibronectin and enhancement of cel-

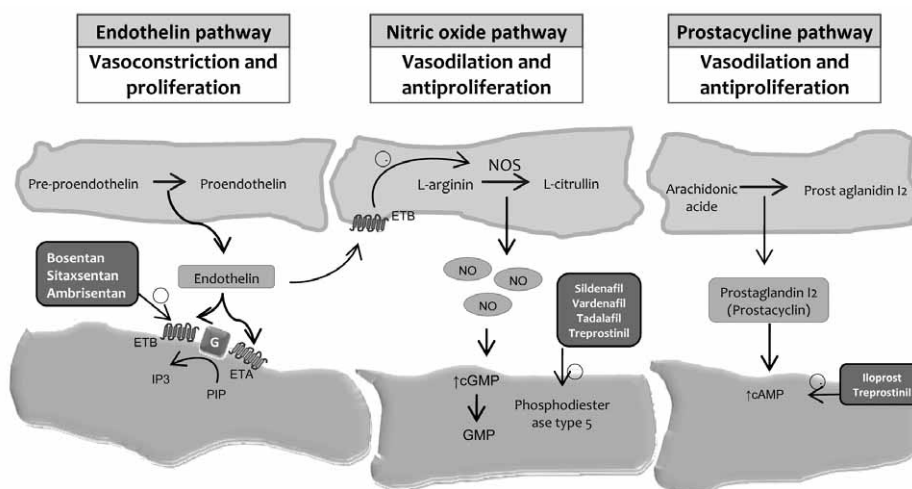


Figure 1. Main pathways involved in the pathophysiology of PAH (based on ref 32).

lular migration (28). This could lead to increased integrin signaling with resulting expression of the glycoprotein tenascin C which acts with other growth factors to enhance smooth muscle proliferation (28). Inhibitors of elastase can induce the apoptosis of smooth muscle cells in cell culture (28).

MODIFICATION OF MEDIATORS AND ITS PATHWAYS IN THE THERAPY OF PAH

PAH is a complex disease which demands multidisciplinary and individual approach. The main focus of PAH therapeutical approach is improvement of symptoms, quality of life, and clinical outcome, as well as prevention of disease progression. All of them include some lifestyle changes, such as plenty of rest, moderate activity, smoking cessation, avoiding of use of birth control pills, and drugs that can lower blood pressure excessively, stress reduction, healthy diet and maintenance of healthy weight (21). Treatment of PAH can be medicamentous and well as surgical, depending on the disease development stage. Surgical treatment includes atrial septostomy, pulmonary endarterectomy (in patients with chronic thromboembolic disease and large central clots) and lung transplantation (21).

Medications include vasodilators, endothelin receptor antagonists, PDE 5 inhibitors, sometimes high dose calcium channel blockers, anticoagulants, diuretics and oxygen. Current drugs include synthetic prostanoids – vasodilators and antiplatelet agents (iloprost, epoprostenil, beraprost, treprostinil) which decrease mean pulmonary artery pressure (PAP) and increase the cardiac index in patients with PAH, prolonging survival (21). Epoprostenol, a synthetic prostacyclin, iloprost and treprostinil – synthetic analogues, are used to treat patients with PAH. These drugs have improved circulatory hemodynamics, breathing, exercise tolerance and survival. The inhalation of these drugs can reduce pulmonary vascular pressure without affecting systemic vascular pressure (22). Prostanoids in combination with other drugs selective for the pulmonary circulation may be a choice in PAH therapy (22). Producing an oral prostanoid is important in the management of PAH, because it will bridge over a number of problems associated with intravenous and central venous catheter use (infection, pump failure, and catheter and pump occlusion) (22).

Phosphodiesterase-5 inhibitors, such as sildenafil, decrease the breakdown of cGMP, increasing intracellular levels of this vasorelaxing agent (24).

Leukotriene receptor antagonist, zafirlukast, decreases pulmonary arterial pressure (PAP) by inhibiting the action of leukotriens and reduces PAP and pulmonary venous pressure, but it does not affect the systemic mean blood pressure (24).

Endothelin receptor blockers, *e.g.* bosentan is an oral nonselective or dual-endothelin receptor antagonist that blocks both ET-A and ET-B receptors. Bosentan decreases pulmonary vascular resistance and improves the results of functional tests (24). It has been established as a

first-line option for the majority of PAH patients. Endothelin – receptor antagonists (ETRAs) comprise sulfonamide and non –sulfonamide agents with different affinities for ET-receptor subtypes (Eta and ETb). Selective ETA-receptor antagonists should be more effective in achieving this than non-selective ETA/ETB – receptor antagonists (25). Selective ETB – receptor blockade results in vasoconstriction and reduced blood flow. Co-administration of selective ETA- and ETB- receptor antagonists attenuates the vasodilator response relative to selective ETA-receptor blockade (25). Classical vasodilators cannot be used to treat PAH because of their systemic hypotensive effects.

Anticoagulants are indicated in thrombosis in situ (found in 25 to 50% of these patients) (25). Calcium-channel blockers may be effective in 5% of patients, prolonging survival. Other treatments include positive airway pressure therapy for obstructive sleep apnea, or oxygen for hypoxemia and surgery: atrial septostomy, pulmonary endarterectomy (in patients with chronic thromboembolic disease and large central clots) and lung transplantation (25). Figure 1 schematically presents main pathways involved in the pathophysiology of PAH and the sites of action of current drugs in PAH treatment (29, 30).

PERSPECTIVES IN THE FUTURE THERAPY OF PAH

There are several drugs in clinical trials, *e.g.* the selective oral ET-A antagonists sitaxsentan and ambrisentan, long-acting oral PDE-5 inhibitors vardenafil or tadalafil, inhaled treprostinil, inhaled vasoactive intestinal peptide, and intravenous or inhaled adrenomedullin (29).

Possible future therapy includes retinoic acid, gene and cell therapy and modification of cell signaling pathways, such as Rho-kinase pathway. Retinoic acid increases the transcription of prostacyclin synthase in endothelial cells and retinoic acid receptors may mediate the production of prostacyclin synthase (31). The future of PAH treatment could involve developing agonists for the retinoic acid receptor, or using retinoic acid to increase the expression of prostacyclin synthase, and consequently prostacyclin production and its beneficial vasodilator effects (31). Gene therapy is based on the delivered genes into the pulmonary vasculature by a viral vector or by another fashion, with the task to produce a desired effect on the vascular wall (31). For example, candidates for gene therapy would be PAH patients with mutation in ALK 1 or endoglin genes (8).

Cell therapy includes the use of cells to increase the production of prostacyclin (32). Mesenchymal stem cells (MSCs) that overexpress prostacyclin synthase (PGIS) have been used in Japan to enhance engraftment and neovascularization in a mouse model of hind-limb ischemia (32) and could be, according to the same principle, used in the cell therapy of PAH. Additionally, future direction could be combination of cell and gene therapy. A previous study has shown that NO produced by endo-

thelial nitric oxide synthase (eNOS) decreases pulmonary arterial pressure in rats. Cell based therapy with eNOS was more effective in treating PAH than cell-based therapy with VEGF (32). Endothelial progenitor cells (EPCs) are responsible for the neovascularization of ischemic tissues and for the repair of the vascular endothelial lining (32). EPCs can be damaged after the development of hypoxia-induced PAH. Endothelial progenitor cells from the umbilical cord have a high capacity for phagocytosis and can be transduced with genes that are therapeutic for PAH (32). Fusion – hybrid enzyme links COX and PGIS together to specifically synthesize prostacyclin from arachidonic acid (32). This hybrid enzyme increases prostacyclin production in the cells transfected with a fusion enzyme gene (32). The combination of the fusion enzyme with cell and gene therapy may result in a more effective approach to PAH treatment.

The development of new prostacyclin receptor agonist drugs is one of the most crucial next steps in the treatment of PAH. A novel agonist test drug selexipag (2-(4(5,6-diphenylpyrazin-2-yl)(isopropyl)amino)butoxy)-N-(methylsulfonyl)acetamide (NS-304), alleviated vascular endothelial dysfunction, pulmonary arterial wall hypertrophy, and RV hypertrophy, and increased RV systolic pressure (24, 33, 34).

Multiple cell types in the vascular wall (endothelial and vascular smooth muscle cells, inflammatory cells, and fibroblasts) rely upon the Rho-kinase signaling pathway for homeostatic function and response to injury (26). Rho is a guanosine – triphosphate (GTP) binding protein that activates its downstream target, Rho-kinase (27). When activated, rho-kinase inhibits myosin phosphates and conversely upregulates the ERM family of kinase (27). Intravenous application of fasudil, a selective Rho-kinase inhibitor, can induce pulmonary vasodilatation and regression of PAH in various animal models, as well as in patients with severe PAH who are refractory to conventional therapies (27).

In conclusion, the goals of current treatment regimens are to improve patients' symptoms, to enhance exercise tolerance, to normalize hemodynamic parameters, to improve quality of life and to extend survival. Although there are some promising drugs in the therapy of PAH, there is a need for developing new ones, together with surgical approaches, in order to increase the survival of the patients with PAH.

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